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(FILE 'HOME' ENTERED AT 10:19:56 ON 07 MAY 2003)

FILE 'WPIDS' ENTERED AT 10:20:06 ON 07 MAY 2003

L1 6631 S TRIGLYCERIDE  
L2 19085 S HYDROGEN PEROXIDE  
L3 43 S FREE GLYCEROL  
L4 5 S L1 AND L2 AND L3

FILE 'USPATFULL' ENTERED AT 10:23:27 ON 07 MAY 2003

L5 22 S L3 AND L1 AND L2

=> log hold

COST IN U.S. DOLLARS

SINCE FILE

ENTRY

4.30

TOTAL

SESSION

44.35

FULL ESTIMATED COST

SESSION WILL BE HELD FOR 60 MINUTES

STN INTERNATIONAL SESSION SUSPENDED AT 10:24:52 ON 07 MAY 2003

1

AN 101:86573 CA

TI An influence of the free **glycerol** on **triglyceride** measurement, and the **triglyceride** measurement by free **glycerol** elimination method

AU Yoshida, Takanori; Takeda, Chikako; Uemura, Taizo; Hayashi, Masaru  
CS Cent. Clin. Res. Lab., Nissei Hosp., Osaka, Japan

SO Nissei Byoin Igaku Zasshi (1984), 12(1), 53-7

CODEN: NBIZDW; ISSN: 0301-2581

DT Journal

LA Japanese

AB For the detn. of serum **triglycerides**, **glycerol** in the sample was removed by treatment with ATP and **glycerol kinase** to form L-**glycerol** 3-phosphate which was treated with L-**glycerol** 3-phosphate **oxidase** to produce H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> produced was treated with Na N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline (ADOS) in the presence of peroxidase to give a colorless product. **Triglycerides** in the sample, then was treated with lipoprotein **lipase** to give **glycerol**, which was treated with **glycerol kinase** and ATP to form L-**glycerol** 3-phosphate. L-**Glycerol** 3-phosphate was further treated with 4-aminoantipyrine and ADOS in the presence of peroxidase to form a product for spectrophotometric detn. Av. free **glycerol** in serum sample from outpatients was 7.0 mg/mL and that in serum samples from inpatients was 8.3 mg/dL. Of 37 outpatients tested, no correlation between **triglyceride** values and **glycerol** values was obsd. Time required for total removal of free **glycerol** was 3-4 min. Bilirubin and **glycerol** almost had no effect on the detn. of **triglycerides**. The method pos. correlated with the GPO method (.gamma. = 0.998).

L21 ANSWER 33 OF 71 CA COPYRIGHT 2003 ACS  
AN 98:212357 CA  
TI Evaluation of a reagent for determination of **triglycerides**  
without overdetermination of free **glycerol**  
AU Ohkubo, Shigeo; Mashige, Fumiko; Kamei, Yukiko; Ohkubo, Akiyuki; Yamanaka,  
Manabu  
CS Fac. Med., Univ. Tokyo, Tokyo, Japan  
SO Rinsho Kensa (1983), 27(3), 329-32  
CODEN: RNKNAT; ISSN: 0485-1420  
DT Journal  
LA Japanese  
AB The detn. of **triglycerides** in a sample contg. free  
**glycerol** by a colorimetric method is based on the reaction of free  
**glycerol** with **glycerol oxidase** in the presence  
of O for the removal of free **glycerol**. As an example, a  
20-.mu.L serum sample was treated with reagent A contg. **glycerol**  
**oxidase**, peroxidase, aldehyde **oxidase**, Good's buffer (pH  
6.75) and N-ethyl-N-(3-methylphenyl)-N'-acetylenediamine (EMAE) at  
37.degree. for 5 h, followed by treatment with reagent B contg.  
lipoprotein **lipase**, peroxidase, 4-aminoantipyrine, Good's buffer  
(pH 6.75) and EMAE and colorimetric anal. at 550 nm for the detn. of  
**triglycerides**. Reproducibility with a relative std. deviation of  
1.0% (intra-assay) and 3.7% (interassay) was obsd. Bilirubin, ascorbic  
acid, GSH and NaN3 at concns. tested had little or no effect on the detn.  
The method was simple and rapid and in good agreement with other methods.

Chemical Fragment Codes (M1):

\*04\* M423 M760 M903 N102 V752 V772  
\*05\* M423 M430 M782 M903 P831 Q233 V802 V811 V813

Chemical Fragment Codes (M2):

\*02\* F011 F012 F013 F014 F015 F512 G010 G100 H1 H100 H121 H2 H212 J5 J521  
L9 L941 M210 M211 M240 M273 M281 M320 M413 M430 M510 M521 M531 M540  
M782 M903 M904 N102 P831 Q233 R03159-D R03159-M  
\*03\* G012 G100 H1 H103 H141 H4 H402 H482 H8 M210 M211 M240 M281 M312 M322  
M332 M342 M383 M392 M414 M430 M510 M520 M531 M540 M782 M903 M904  
N102 P831 Q233 R20837-D R20837-M

Chemical Fragment Codes (M5):

\*01\* M750 M903 M904 M910 N102 Q233 S005 S032 S131 S133 S134 S142 S143  
S303 S317 S503 U560 U563 R00148-A

Chemical Fragment Codes (M6):

\*06\* M903 P831 R511 R515 R520 R521 R624 R638

Derwent Registry Numbers: 0148-U

Specific Compound Numbers: R00148-A; R03159-D; R03159-M; R20837-D; R20837-M

6/9/2 (Item 1 from file: 345)

DIALOG(R) File 345: Inpadoc/Fam. & Legal Stat

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16673914

Basic Patent (No, Kind, Date): CA 2185562 AA 19960808 <No. of Patents: 017>

PATENT FAMILY:

AUSTRIA (AT)

Patent (No, Kind, Date): AT 197070 E 20001115

VERFAHREN ZUR QUANTITATIVEN ANALYSE VON CHOLESTERIN (German)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)

Author (Inventor): HINO KOICHI; NAKAMURA MITSUHIRO; MANABE MITSUHIRO

Priority (No, Kind, Date): JP 9513607 A 19950131; WO 95JP641 W  
19950403

Applic (No, Kind, Date): EP 95913411 A 19950403

Addnl Info: 753583 20001018

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: German

AUSTRIA (AT)

Legal Status (No, Type, Date, Code, Text):

AT 197070 R 20001115 AT REF CORRESPONDS TO EP-PATENT  
(ENTSPRICHT EP-PATENT)

EP 753583 P 20001018

AT 197070 R 20010215 AT UEP PUBLICATION OF TRANSLATION  
OF EUROPEAN PATENT SPECIFICATION  
(UEBERSETZUNG DER EUROPÄISCHEN PATENTSCHRIFT  
AUSGEGEBEN)

AUSTRALIA (AU)

Patent (No, Kind, Date): AU 9520852 A1 19960821

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD

Author (Inventor): HINO KOICHI; NAKAMURA MITSUHIRO; MANABE MITSUHIRO

Priority (No, Kind, Date): JP 9513607 A 19950131; WO 95JP641 W  
19950403

Applic (No, Kind, Date): AU 9520852 A 19950403

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: English

Patent (No, Kind, Date): AU 696681 B2 19980917

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD

Author (Inventor): HINO KOICHI; NAKAMURA MITSUHIRO; MANABE MITSUHIRO

Priority (No, Kind, Date): JP 9513607 A 19950131; WO 95JP641 W

19950403  
Applic (No,Kind,Date): 9520852 A 19950403  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: English

CANADA (CA)

Patent (No,Kind,Date): CA 2185562 AA 19960808  
METHOD FOR QUANTITATIVELY DETERMINING CHOLESTEROL (English; French)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): CA 2185562 A 19950403  
IPC: \* C12Q-001/60  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: English

CANADA (CA)

Legal Status (No,Type,Date,Code,Text):  
CA 2185562 P 19960913 CA REFW CORRESPONDS TO PCT  
APPLICATION (ENTSPRICHT PCT ANMELDUNG)  
WO 9623902 P

CHINA (CN)

Patent (No,Kind,Date): CN 1145096 A 19970312  
METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): KOUICHI HINO (JP); MITSUHIRO NAKAMURA (JP); MITSUHIRO MANABE (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): CN 95192343 A 19950403  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: Chinese

GERMANY (DE)

Patent (No,Kind,Date): DE 69519160 C0 20001123  
VERFAHREN ZUR QUANTITATIVEN ANALYSE VON CHOLESTERIN (German)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): HINO KOICHI ( ); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131; WO 95JP641 W 19950403  
Applic (No,Kind,Date): DE 69519160 A 19950403  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: German  
Patent (No,Kind,Date): DE 69519160 T2 20010517  
VERFAHREN ZUR QUANTITATIVEN ANALYSE VON CHOLESTERIN (German)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): HINO KOICHI ( ); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131; WO 95JP641 W 19950403  
Applic (No,Kind,Date): DE 69519160 A 19950403  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: German

GERMANY (DE)

Legal Status (No,Type,Date,Code,Text):  
DE 69519160 P 20001123 DE REF CORRESPONDS TO (ENTSPRICHT)

DE 69519160

P

200

EP 753583 P 20001123

TRANSLATION OF PATENT

DOCUMENT OF EUROPEAN PATENT HAS BEEN RECEIVED AND  
HAS BEEN PUBLISHED (UEBERSETZUNG DER  
PATENTSCHRIFT DES EUROPÄISCHEN PATENTES IST  
EINGEGANGEN UND VERÖFFENTLICHT WORDEN)

DENMARK (DK)

Patent (No,Kind,Date): DK 753583 T3 20001113

FREMGANGSMAADE TIL KVANTITATIV ANALYSE AF CHOLESTEROL (Danish)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)

Author (Inventor): HINO KOICHI (JP); IICHI PURE (JP); NAKAMURA  
MITSUHIRO (JP); MANABE MITSUHIRO (JP)

Priority (No,Kind,Date): JP 9513607 A 19950131; WO 95JP641 W  
19950403

Applic (No,Kind,Date): DK 9595913411 A 19950403

IPC: \* C12Q-001/60; C12Q-001/26; C12Q-001/44; G01N-033/92

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: Danish

EUROPEAN PATENT OFFICE (EP)

Patent (No,Kind,Date): EP 753583 A1 19970115

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English; French;  
German)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)

Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE  
MITSUHIRO (JP)

Priority (No,Kind,Date): WO 95JP641 W 19950403; JP 9513607 A  
19950131

Applic (No,Kind,Date): EP 95913411 A 19950403

Designated States: (National) AT; BE; CH; DE; DK; ES; FR; GB; GR; IE;  
IT; LI; LU; MC; NL; PT; SE

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: English

Patent (No,Kind,Date): EP 753583 A4 19971210

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English; French;  
German)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)

Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE  
MITSUHIRO (JP)

Priority (No,Kind,Date): WO 95JP641 W 19950403; JP 9513607 A  
19950131

Applic (No,Kind,Date): EP 95913411 A 19950403

Designated States: (National) AT; BE; CH; DE; DK; ES; FR; GB; GR; IE;  
IT; LI; LU; MC; NL; PT; SE

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: English

Patent (No,Kind,Date): EP 753583 B1 20001018

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL (English; French;  
German)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)

Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE  
MITSUHIRO (JP)

Priority (No,Kind,Date): WO 95JP641 W 19950403; JP 9513607 A  
19950131

Applic (No,Kind,Date): EP 95913411 A 19950403

Designated States: (National) AT; BE; CH; DE; DK; ES; FR; GB; GR; IE;  
IT; LI; LU; MC; NL; PT; SE

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92

CA Abstract No: \* 125(17)216395E

Derwent WPI Acc No: \* C 96-371447

Language of Document: English

## EUROPEAN PATENT OFFICE (EP)

Legal Status (No, Type, Date, Code, Text):

EP 753583

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19950131

EP AA

PRIORITY (PATENT)

APPLICATION) (PRIORITAET (PATENTANMELDUNG))

EP 753583

P

19950403

EP AA

JP 9513607 A 19950131

PCT-APPLICATION

(PCT-ANMELDUNG)

EP 753583

P

19950403

EP AE

WO 95JP641 W 19950403

EP-APPLICATION

(EUROPAEISCHE ANMELDUNG)

EP 753583

P

19970115

EP AK

EP 95913411 A 19950403

DESIGNATED CONTRACTING

STATES IN AN APPLICATION WITH SEARCH REPORT:  
(IN EINER ANMELDUNG BENANNT VERTRAGSSTAATEN)

EP 753583

P

19970115

EP A1

AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL

PT SE

PUBLICATION OF APPLICATION

WITH SEARCH REPORT (VEROEFFENTLICHUNG DER

ANMELDUNG MIT RECHERCHENBERICHT)

EP 753583

P

19970115

EP 17P

REQUEST FOR EXAMINATION

FILED (PRUEFUNGSANTRAG GESTELLT)

960905

EP 753583

P

19971210

EP AK

DESIGNATED CONTRACTING

STATES MENTIONED IN A SUPPLEMENTARY SEARCH

REPORT: (IN EINEM ERGAENZENDEN

RECHERCHENBERICHT BENANNT VERTRAGSSTAATEN)

EP 753583

P

19971210

EP A4

AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL

PT SE

SUPPLEMENTARY SEARCH REPORT

(ERGAENZENDER RECHERCHENBERICHT)

EP 753583

P

19990818

EP 17Q

FIRST EXAMINATION REPORT

(ERSTER PRUEFUNGSBESCHEID)

19990705

EP 753583

P

20000308

EP 17Q

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(ERSTER PRUEFUNGSBESCHEID)

19990705

EP 753583

P

20001018

EP AK

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STATES MENTIONED IN A PATENT SPECIFICATION:

(IN EINER PATENTSCHRIFT ANGEFUEHRTE BENANNT

VERTRAGSSTAATEN)

AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL

PT SE

EP 753583

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20001018

EP B1

PATENT SPECIFICATION

(PATENTSCHRIFT)

EP 753583

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20001018

EP REF

IN AUSTRIA REGISTERED AS:

(IN AT EINGETRAGEN ALS:)

AT 197070 R 20001115

EP 753583

P

20001031

CH EP/REG

ENTRY IN THE NATIONAL PHASE

(EINTRITT IN DIE NATIONALE PHASE)

EP 753583

P

20001113

DK T3/REG

TRANSLATION OF EP PATENT

EP 753583

P

20001115

IE FG4D/REG

EUROPEAN PATENTS GRANTED

DESIGNATING IRELAND

EP 753583

P

20001115

IE FG4D/REG

EUROPEAN PATENTS GRANTED

DESIGNATING IRELAND

EP 753583

P

20001123

EP REF

CORRESPONDS TO:

(ENTSPRICHT)

DE 69519160 P 20001123

EP 753583

P

20001124

EP ET

FR: TRANSLATION FILED (FR:

TRADUCTION A ETE REMISE)

EP 753583

P

20001215

EP ITF

IT: TRANSLATION FOR A EP

PATENT FILED (IT: DEPOSITO TRADUZIONE DI

BREVETTO EUROPEO)

SOCIETA' ITALIANA BREVETTI S.P.A.

EP 753583

P

20010216

ES FG2A/REG

DEFINITIVE PROTECTION

(PROTECCION DEFINITIVA)

## SPAIN (ES)

Patent (No,Kind,Date): ES 2153030 T3 20010216  
PROCEDIMIENTO DE ANALISIS CUANTITATIVO DEL COLESTEROL. (Spanish)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD  
Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): ES 95913411 EP 19950403  
Addnl Info: 753583 EP patent valid in AT  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: Spanish

## SPAIN (ES)

Legal Status (No,Type,Date,Code,Text):  
ES 2153030 P 20010216 ES FG2A DEFINITIVE PROTECTION  
(PROTECCION DEFINITIVA)  
753583

## JAPAN (JP)

Patent (No,Kind,Date): JP 8201393 A2 19960809  
QUANTIFYING METHOD FOR CHOLESTEROL (English)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD  
Author (Inventor): HINO KOICHI; NAKAMURA MITSUHIRO; MANABE MITSUHIRO  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): JP 9513607 A 19950131  
IPC: \* G01N-033/92; G01N-033/53  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: Japanese  
Patent (No,Kind,Date): JP 2799835 B2 19980921  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): JP 9513607 A 19950131  
IPC: \* C12Q-001/60; C12Q-001/26; C12Q-001/44; G01N-033/53; G01N-033/92  
Language of Document: Japanese

## PORTUGAL (PT)

Patent (No,Kind,Date): PT 753583 T 20010330  
METODO PARA ANALISE QUANTITATIVA DO COLESTEROL (English; French; German ; Portugese)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); CO MANABE MITSUHIRO DAIICHI PU (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131  
Applic (No,Kind,Date): PT 95913411 A 19950403  
IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26; G01N-033/92  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: Portugese

## UNITED STATES OF AMERICA (US)

Patent (No,Kind,Date): US 5773304 A 19980630  
METHOD FOR QUANTITATIVELY DETERMINING CHOLESTEROL (English)  
Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP)  
Author (Inventor): HINO KOICHI (JP); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)  
Priority (No,Kind,Date): JP 9513607 A 19950131; WO 95JP641 W 19950403  
Applic (No,Kind,Date): US 704681 A 19960919  
National Class: \* 436174000; 435007100; 435007800; 435007910;  
435011000; 435019000; 435962000; 436174000; 436539000; 436013000;  
436017000; 436063000; 436071000; 436815000; 436824000; 436825000;  
436826000



IPC: \* G01N-033/533  
CA Abstract No: \* 125(17)216395E  
Derwent WPI Acc No: \* C 96-371447  
Language of Document: English

UNITED STATES OF AMERICA (US)

Legal Status (No,Type,Date,Code,Text):

US 96704681 A 19960919 US REFW CORRESPONDS TO PCT  
APPLICATION (ENTSPRICHT PCT ANMELDUNG)  
WO 9623902 P  
US 5773304 P 19950131 US AA PRIORITY (PATENT)  
JP 9513607 A 19950131  
US 5773304 P 19950403 US AA PCT-APPLICATION (PCT-APPL.)  
WO 95JP641 W 19950403  
US 5773304 P 19960919 US AE APPLICATION DATA (PATENT)  
(APPL. DATA (PATENT))  
US 704681 A 19960919  
US 5773304 P 19961024 US AS02 ASSIGNMENT OF ASSIGNOR'S  
INTEREST  
DAIICHI PURE CHEMICALS, CO., LTD. 13-5,  
NIHOMBASHI 3-CHOME, CHUO-KU TOKYO 103, J ;  
HINO, KOICHI : 19960826; NAKAMURA, MITSUHIRO  
: 19960826; MANABE, MITSUHIRO : 19960826  
US 5773304 P 19980630 US A PATENT

WORLD INTELLECTUAL PROPERTY ORGANIZATION, PCT (WO)

Patent (No,Kind,Date): WO 9623902 A1 19960808

METHOD OF QUANTITATIVE ANALYSIS OF CHOLESTEROL METHOD OF QUANTITATIVE  
ANALYSIS OF CHOLESTEROL (English)

Patent Assignee: DAIICHI PURE CHEMICALS CO LTD (JP); HINO KOUICHI  
(JP); NAKAMURA MITSUHIRO (JP); MANABE MITSUHIRO (JP)

Author (Inventor): HINO KOUICHI (JP); NAKAMURA MITSUHIRO (JP);  
MANABE MITSUHIRO (JP)

Priority (No,Kind,Date): JP 9513607 A 19950131

Applic (No,Kind,Date): WO 95JP641 A 19950403

Designated States: (National) AU; CA; CN; KR; MX; US (Regional) AT;  
BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE

Filing Details: WO 100000 With international search report

IPC: \* C12Q-001/60; C12Q-001/44; C12Q-001/26

CA Abstract No: \* 125(17)216395E; 125(17)216395E

Derwent WPI Acc No: \* C 96-371447; C 96-371447

Language of Document: Japanese

WORLD INTELLECTUAL PROPERTY ORGANIZATION, PCT (WO)

Legal Status (No,Type,Date,Code,Text):

WO 9623902 P 19950131 WO AA PRIORITY (PATENT)  
JP 9513607 A 19950131  
WO 9623902 P 19950403 WO AE APPLICATION DATA (APPL.  
DATA)  
WO 95JP641 A 19950403  
WO 9623902 P 19960808 WO AK DESIGNATED STATES CITED IN A  
PUBLISHED APPLICATION WITH SEARCH REPORT  
(DESIGNATED STATES CITED IN A PUBLISHED APPL.  
WITH SEARCH REPORT)  
AU CA CN KR MX US  
WO 9623902 P 19960808 WO AL DESIGNATED COUNTRIES FOR  
REGIONAL PATENTS CITED IN A PUBLISHED  
APPLICATION WITH SEARCH REPORT (DESIGNATED  
COUNTRIES FOR REGIONAL PATENTS CITED IN A  
PUBLISHED APPL. WITH SEARCH REPORT)  
AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT  
SE  
WO 9623902 P 19960808 WO A1 PUBLICATION OF THE  
INTERNATIONAL APPLICATION WITH THE  
INTERNATIONAL SEARCH REPORT (PUB. OF THE  
INTERNATIONAL APPL. WITH THE INTERNATIONAL  
SEARCH REPORT)

AN 102:163371 CA  
TI Quantitative determination of **triglycerides**  
PA Wako Pure Chemical Industries, Ltd., Japan  
SO Jpn. Kokai Tokkyo Koho, 10 pp.  
CODEN: JKXXAF  
DT Patent  
LA Japanese  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 60024200	A2	19850206	JP 1983-130546	19830718
PRAI	JP 1983-130546		19830718		

AB In the detn. of **triglycerides** in biol. samples, free **glycerol** in a sample is converted to **glycerol** 3-phosphate with **glycerol kinase** in the presence of ATP, and **triglycerides** are hydrolyzed to fatty acids and **glycerol**, which is oxidized by **glycerol oxidase** to form H<sub>2</sub>O<sub>2</sub> for the quant. detn. of **triglycerides**. Thus, a 20- $\mu$ L std. or blood serum sample was treated with 0.5 mL of a reagent contg. 0.1M Tris buffer (pH 6.85), ATP, **glycerol kinase**, and Mg acetate at 37.degree. for 5 min and then with 0.5 mL of a reagent contg. 0.1M Tris buffer (pH 7.0), ADP, p-chlorophenol, 4-aminoantipyrine, lipoprotein **lipase**, **glycerol oxidase**, and peroxidase at 37.degree. for 10 min, and the reaction mixt. was analyzed at 505 nm for the detn. of **triglycerides**.

*no inhibition  
of H<sub>2</sub>O<sub>2</sub> in  
first step*

L21 ANSWER 26 OF 71 CA COPYRIGHT 2003 ACS

AN 101:86573 CA

TI An influence of the free **glycerol** on **triglyceride** measurement, and the **triglyceride** measurement by free **glycerol** elimination method

AU Yoshida, Takanori; Takeda, Chikako; Uemura, Taizo; Hayashi, Masaru

CS Cent. Clin. Res. Lab., Nissei Hosp., Osaka, Japan

SO Nissei Byoin Igaku Zasshi (1984), 12(1), 53-7

CODEN: NBIZDW; ISSN: 0301-2581

DT Journal

LA Japanese

AB For the detn. of serum **triglycerides**, **glycerol** in the sample was removed by treatment with ATP and **glycerol kinase** to form L-**glycerol** 3-phosphate which was treated with L-**glycerol** 3-phosphate **oxidase** to produce H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> produced was treated with Na N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline (ADOS) in the presence of peroxidase to give a colorless product. **Triglycerides** in the sample then was treated with lipoprotein **lipase** to give **glycerol**, which was treated with **glycerol kinase** and ATP to form L-**glycerol** 3-phosphate. L-**Glycerol** 3-phosphate was further treated with 4-aminoantipyrine and ADOS in the presence of peroxidase to form a product for spectrophotometric detn. Av. free **glycerol** in serum sample from outpatients was 7.0 mg/mL and that in serum samples from inpatients was 8.3 mg/dL. Of 37 outpatients tested, no correlation between **triglyceride** values and **glycerol** values was obsd. Time required for total removal of free **glycerol** was 3-4 min. Bilirubin and **glycerol** almost had no effect on the detn. of **triglycerides**. The method pos. correlated with the GPO method (.gamma. = 0.998).

L21 ANSWER 27 OF 71 CA COPYRIGHT 2003 ACS

AN 100:171187 CA

TI Reagents for the determination of **triglycerides**

PA Toyobo Co., Ltd., Japan

SO Jpn. Kokai Tokkyo Koho, 6 pp.

CODEN: JKXXAF

DT Patent

PI JP 58067196 A2 19830421 JP 1981-163469 19811015  
 PRAT JP 1981-163469 19811015  
 AB An enzymic-spectrophotometric assay for **triglycerides** is described which uses a Good's buffer contg. polyoxyethylene 3,5,5-tetramethylhexanol (I) as detergent. For example, a reagent was prepd. which contained lipoprotein **lipase**, **glycerol kinase**, **glycerol 3-phosphate oxidase**, peroxidase, 4-aminoantipyrine, ATP, N-(2-carboxyl)-3-methylaniline, MgCl<sub>2</sub>, and I in 50 mM Bicine (pH 7.6). This reagent (3.0 mL) was added to 20 .mu.L serum, then incubated at 37.degree. for 5 min. The H<sub>2</sub>O<sub>2</sub> formed was measured at 550 nm. The method was 3-fold faster and the color produced was more stable than in a conventional method.

L21 ANSWER 33 OF 71 CA COPYRIGHT 2003 ACS  
 AN 98:212357 CA  
 TI Evaluation of a reagent for determination of **triglycerides** without overdetermination of free **glycerol**  
 AU Ohkubo, Shigeo; Mashige, Fumiko; Kamei, Yukiko; Ohkubo, Akiyuki; Yamanaka, Manabu  
 CS Fac. Med., Univ. Tokyo, Tokyo, Japan  
 SO Rinsho Kensa (1983), 27(3), 329-32  
 CODEN: RNKNAT; ISSN: 0485-1420  
 DT Journal  
 LA Japanese  
 AB The detn. of **triglycerides** in a sample contg. free **glycerol** by a colorimetric method is based on the reaction of free **glycerol** with **glycerol oxidase** in the presence of O for the removal of free **glycerol**. As an example, a 20-.mu.L serum sample was treated with reagent A contg. **glycerol oxidase**, peroxidase, aldehyde **oxidase**, Good's buffer (pH 6.75) and N-ethyl-N-(3-methylphenyl)-N'-acetylenediamine (EMAE) at 37.degree. for 5 h, followed by treatment with reagent B contg. lipoprotein **lipase**, peroxidase, 4-aminoantipyrine, Good's buffer (pH 6.75) and EMAE and colorimetric anal. at 550 nm for the detn. of **triglycerides**. Reproducibility with a relative std. deviation of 1.0% (intra-assay) and 3.7% (interassay) was obsd. Bilirubin, ascorbic acid, GSH and Na<sub>2</sub>S at concns. tested had little or no effect on the detn. The method was simple and rapid and in good agreement with other methods.

L21 ANSWER 34 OF 71 CA COPYRIGHT 2003 ACS  
 AN 98:157264 CA  
 TI A peroxidase-coupled method for the colorimetric determination of serum **triglycerides**  
 AU McGowan, Michael W.; Artiss, Joseph D.; Strandbergh, Donald R.; Zak, Bennie  
 CS Sch. Med., Wayne State Univ., Detroit, MI, 48201, USA  
 SO Clinical Chemistry (Washington, DC, United States) (1983), 29(3), 538-42  
 CODEN: CLCHAU; ISSN: 0009-9147  
 DT Journal  
 LA English  
 AB An enzymic method for rapid, precise measurement of blood serum **triglycerides** with use of sample/reagent ratios as large as 1:200 is described. Hydrolysis of **triglycerides** is catalyzed by **lipase** to produce **glycerol** and free fatty acids. The **glycerol** generated is then phosphorylated by ATP in the presence of **glycerol kinase**. Oxidn. of the resulting **glycerol 3-phosphate** to produce H<sub>2</sub>O<sub>2</sub> is catalyzed by L-.alpha.-glycerophosphate **oxidase**. An intense red chromogen is produced by the peroxidase-catalyzed coupling of 4-aminoantipyrine and Na 2-hydroxy-3,5-dichlorobenzenesulfonate with H<sub>2</sub>O<sub>2</sub>. This sensitive chromogen system not only permits use of unusually small sample vols., it also facilitates a linear response to blood serum **triglyceride** concns. up to at least 10 g/L while displaying good Ringbom (measure of accuracy) characteristics.

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 59028664	A2	19840215	JP 1982-139499	19820811
	JP 01034350	B4	19890719		
PRAI	JP 1982-139499		19820811		

AB Reagents for the enzymic-photometric detn. of **triglycerides** consist of lipoprotein **lipase**, **glycerol kinase**, **glycerophosphate oxidase** or **glycerol oxidase**, peroxidase, coupling agents, and aniline derivs. with addn. of ferrocyanide (0.1-1.0  $\mu\text{mol/L}$ ). Unlike conventional methods, bilirubin in the samples does not interfere with the anal. Thus, serum samples were treated with a reagent contg. Tris buffer (pH 7.5), lipoprotein **lipase** (200 units/mL), **glycerol kinase** (1 unit/mL), **glycerophosphate oxidase** (2.5 units/mL), peroxidase (7.5 units/mL), 4-aminoantipyrine (3 mg/dL), N,N-dimethyl-m-toluidine (20 mg/dL), and K ferrocyanide (0.30  $\mu\text{mol/L}$ ) at 37.degree. for 10 min, and reaction mixt. was analyzed photometrically at 545 nm for the detn. of serum **triglycerides**.

L21 ANSWER 28 OF 71 CA COPYRIGHT 2003 ACS

AN 100:152896 CA

TI **Glycerol oxidase**, a novel copper hemoprotein from *Aspergillus japonicus*. Molecular and catalytic properties of the enzyme and its application to the analysis of serum **triglycerides**

AU Uwajima, Takayuki; Shimizu, Yoshiaki; Terada, Osamu

CS Tokyo Res. Lab., Kyowa Hakko Kogyo Co., Machida, 194, Japan

SO Journal of Biological Chemistry (1984), 259(5), 2748-53

CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

AB Purified **glycerol oxidase** (I) of *A. japonicus* AT 008 had a mol. wt. of 400,000 and contained 1 mol of protoheme IX and 2 g-atoms Cu/mol I protein. The absorption max. of the oxidized form were at 557, 530, 420, 280, and 238 nm; those of the reduced form were at 557 and 430 nm. Anaerobic addn. of **glycerol** to I produced both a shift in the Soret band from 420 to 410 nm and bleaching of the .alpha. and .beta. bands at 557 and 530 nm. The ESR spectrum of I showed 3 major signals at  $g = 1.99$ ,  $2.00$ , and  $2.02$ . The signals at  $g = 1.99$  and  $2.02$  were diminished by the anaerobic addn. of **glycerol**, and the 3 signals completely disappeared after the addn. of either dithionite or diethyldithiocarbamate. Exposure of I to borate buffer of pH 10.0 resulted in activation of I with concomitant enhancement of the ESR signals at  $g = 1.99$  and  $2.02$ . Since I acts predominantly on **glycerol**, it was employed in a specific colorimetric assay for serum **triglycerides** in combination with lipoprotein **lipase**. The **triglyceride** assay results from this method agreed with those obtained by the acetylacetone method.

L21 ANSWER 29 OF 71 CA COPYRIGHT 2003 ACS

AN 99:172271 CA

TI Application of the titanium(IV)-4-(2-pyridylazo)resorcinol reagent to the assay for biological substances. A spectrophotometric method for the determination of **triglycerides** in serum using lipoprotein **lipase**, acyl CoA synthetase and acyl CoA **oxidase**

AU Matsubara, Chiyo; Nishikawa, Yuji; Takamura, Kiyoko

CS Tokyo Coll. Pharm., Tokyo, Japan

SO Yakugaku Zasshi (1983), 103(8), 884-8

CODEN: YKKZAJ; ISSN: 0031-6903

DT Journal

LA Japanese

AB A mixt. of Ti(IV) and 4-(2-pyridylazo)resorcinol (Ti-PAR reagent) is useful for the spectrophotometric detn. of trace amts. of  $\text{H}_2\text{O}_2$ . The

absorbance at 508 nm was proportional to the concn. of H<sub>2</sub>O<sub>2</sub> added. The reagent was successfully applied to the assay of **triglycerides** in serum. **Triglycerides** are enzymically hydrolyzed to produce **glycerol** and fatty acids. The fatty acids were detd. by the detection of H<sub>2</sub>O<sub>2</sub> using the Ti-PAR reagent by the combined use of acyl-CoA synthetase and acyl-CoA **oxidase**. It was possible to det. **triglycerides** in 5 .mu.L serum ranging in concn. 5-600 mg/100 mL. The relative std. deviation was <2.5%. The anal. recovery of **triglycerides** in human serum was >97%. By this method, the absorbance was hardly affected in the presence of reducible substances such as CoA and ascorbic acid.

L21 ANSWER 30 OF 71 CA COPYRIGHT 2003 ACS

AN 99:154590 CA

TI Effect of intravenous infusion of **glycerol** on enzymic assay of serum **triglyceride**

AU Sonoda, Shingo; Katoh, Atsuko; Kasuga, Shinji; Tsuchiya, Fukiko; Kimata, Eriko; Kageyama, Nobuo

CS Dep. Clin. Lab., Chukyo Hosp. Health Insur., Aichi, Japan

SO Rinsho Kensa (1983), 27(7), 809-11

CODEN: RNKNAT; ISSN: 0485-1420

DT Journal

LA Japanese

AB Effects of i.v. infusion of a soln. contg. **glycerol** on the detn. of serum **triglycerides** (neutral lipids) by com. test kits based on enzymic or other reactions were studied. The lipoprotein **lipase**-tetrazolium method and the lipoprotein **lipase**-glucose **oxidase**-peroxidase method (that are not calibrated by serum blank tests) showed a higher value (due to the presence of **glycerol**). The clearance of **glycerol** from blood of patients after i.v. infusion required >3 h. Thus, the time for blood sampling was crit. for accurate anal. The lipoprotein **lipase**-2,4-dinitrophenol method and the lipoprotein **lipase**-GPOD-peroxidase method (that are calibrated by serum blank tests) were not affected by **glycerol**.

L21 ANSWER 31 OF 71 CA COPYRIGHT 2003 ACS

AN 99:136273 CA

TI Comparative study of a new **triglyceride** dye test (GPO-PAP) with an enzymic UV method

AU Richter, Rolf

CS Stolberg, Fed. Rep. Ger.

SO MTA-Journal (1983), 5(7), 294, 296

CODEN: MTJODH; ISSN: 0171-8037

DT Journal

LA German

AB Serum **triglycerides** were detd. with **lipase**, **glycerol kinase**, **glycerol** 3-phosphate **oxidase** (GPO), peroxidase, and 4-aminoantipyrine (PAP), with detection at 546 nm. Results were related to those detd. by a UV method (r = 0.99). Intra- and interassay relative std. deviations were 0.9 and 2.4% resp. Linearity was obsd. up to 1000 mg/dL.

L21 ANSWER 32 OF 71 CA COPYRIGHT 2003 ACS

AN 99:101948 CA

TI Determination of **triglyceride**

PA Shino Test Kenkyusho K. K., Japan

SO Jpn. Kokai Tokkyo Koho, 3 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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L11 ANSWER 12 OF 23 CA COPYRIGHT 2003 ACS

AN 98:212562 CA

TI Process and reagents for the selective **separation** of low-density **lipoproteins** (LDL) and for the quantification of their components

IN Trouyez, Gerard; Alcindor, Louis Gerald

PA Biomerieux S. A., Fr.

SO Eur. Pat. Appl., 11 pp.

CODEN: EPXXDW

DT Patent

LA French

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 76211	A2	19830406	EP 1982-401735	19820927
	EP 76211	A3	19830601		
	EP 76211	B1	19870527		
	R: AT, BE, CH, DE, GB, IT, LI, LU, NL, SE				
	FR 2513766	A1	19830401	FR 1981-18220	19810928
	FR 2513766	B1	19850913		
	ES 515987	A1	19831101	ES 1982-515987	19820927
	AT 27495	E	19870615	AT 1982-401735	19820927
	ES 524070	A1	19840416	ES 1983-524070	19830712
PRAI	FR 1981-18220		19810928		
	EP 1982-401735		19820927		

AB A method is described for the selective pptn. of LDL and for the detn. of their components (cholesterol, apolipoproteins, triglycerides, phospholipids) in body fluids and tissues without pptn. of high-d. or very-low-d. lipoproteins by using a reagent contg. **polyanions**, e.g., PEG or heparin, with or without divalent cations, a detergent, aryl or alkyl ethers of alc. polymers, fatty acid salts, and bile acid salts. The ppt. then is sepd. by centrifugation, dissolved by chelating agents, and the LDL are detd. by turbidimetry or nephelometry. Thus, LDL were isolated from serum by using a pptg. reagent contg. heparin, MnCl<sub>2</sub> (12-120 mM), PEG 6000 or Triton X 100 (0.5-10.0 g/L), and a bile salt (0.5-5 g/L). The ppt. was solubilized with a soln. contg. 0.15M NaCl and EDTA or Na citrate.

IC G01N033-92

CC 9-9 (Biochemical Methods)

ST lipoprotein low density pptn analysis; body fluid lipoprotein pptn analysis; tissue low density lipoprotein pptn

IT Glycerides, analysis

Phospholipids

RL: ANT (Analyte); ANST (Analytical study)

(detn. of, in low-d. lipoproteins, reagent for low-d. lipoproteins pptn. in)

IT Chelating agents

(in low-d. lipoproteins pptn. from body fluids and tissues)

IT Animal tissue

Body fluid

(low-d. lipoproteins of, pptn. of, anal. in relation to)

IT Precipitation

(of low-d. lipoproteins, of body fluids and tissues)

IT Lipoproteins

RL: ANT (Analyte); ANST (Analytical study)

(apo-, detn. of, in low-d. lipoproteins, reagent for low-d. lipoproteins pptn. in)

IT Lipoproteins

RL: ANST (Analytical study)

(low-d., pptn. of, of biol. fluids and tissues, anal. in relation to)

IT 57-88-5, analysis 57-88-5D, esters

RL: ANT (Analyte); ANST (Analytical study)

(detn. of, in low-d. lipoproteins, reagent for low-d. lipoproteins pptn. in)

IT 9002-93-1 60-00-4, uses and miscellaneous 67-42-5 68-04-2 142-73-4  
RL: ANST (Analytical study)  
(in low-d. lipoproteins pptn. from body fluids and tissues)

IT 1180-95-6 7786-30-3, uses and miscellaneous 12067-99-1 25322-68-3  
51312-42-6 9005-49-6, uses and miscellaneous 9042-14-2 10043-52-4,  
uses and miscellaneous  
RL: ANST (Analytical study)  
(pptg. reagent contg., for low-d. lipoproteins pptn. from body fluids  
and tissues)

IT 11132-78-8  
RL: ANST (Analytical study)  
(pptg. reagent contg., for low-d. lipoproteins pptn. in body fluids and  
tissues)

L21 ANSWER 33 OF 71 CA COPYRIGHT 2003 ACS  
AN 98:212357 CA  
TI Evaluation of a reagent for determination of triglycerides  
without overdetermination of free glycerol  
AU Ohkubo, Shigeo; Mashige, Fumiko; Kamei, Yukiko; Ohkubo, Akiyuki; Yamanaka,  
Manabu  
CS Fac. Med., Univ. Tokyo, Tokyo, Japan  
SO Rinsho Kensa (1983), 27(3), 329-32  
CODEN: RNKNAT; ISSN: 0485-1420  
DT Journal  
LA Japanese  
AB The detn. of triglycerides in a sample contg. free  
glycerol by a colorimetric method is based on the reaction of free  
glycerol with glycerol oxidase in the presence  
of O for the removal of free glycerol. As an example, a  
20-.mu.L serum sample was treated with reagent A contg. glycerol  
oxidase, peroxidase, aldehyde oxidase, Good's buffer (pH  
6.75) and N-ethyl-N-(3-methylphenyl)-N'-acetylenediamine (EMAE) at  
37.degree. for 5 h, followed by treatment with reagent B contg.  
lipoprotein lipase, peroxidase, 4-aminoantipyrine, Good's buffer  
(pH 6.75) and EMAE and colorimetric anal. at 550 nm for the detn. of  
triglycerides. Reproducibility with a relative std. deviation of  
1.0% (intra-assay) and 3.7% (interassay) was obsd. Bilirubin, ascorbic  
acid, GSH and NaN3 at concns. tested had little or no effect on the detn.  
The method was simple and rapid and in good agreement with other methods.

Trans sent 8/12/03



AN 101:86573 CA

TI An influence of the free **glycerol** on **triglyceride** measurement, and the **triglyceride** measurement by free **glycerol** elimination method

AU Yoshida, Takanori; Takeda, Chikako; Uemura, Taizo; Hayashi, Masaru  
CS Cent. Clin. Res. Lab., Nissei Hosp., Osaka, Japan

SO Nissei Byoin Igaku Zasshi (1984), 12(1), 53-7  
CODEN: NBIZDW; ISSN: 0301-2581

DT Journal

LA Japanese

AB For the detn. of serum **triglycerides**, **glycerol** in the sample was removed by treatment with ATP and **glycerol kinase** to form L-**glycerol** 3-phosphate which was treated with L-**glycerol** 3-phosphate **oxidase** to produce H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> produced was treated with Na N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline (ADOS) in the presence of peroxidase to give a colorless product. **Triglycerides** in the sample, then was treated with lipoprotein **lipase** to give **glycerol**, which was treated with **glycerol kinase** and ATP to form L-**glycerol** 3-phosphate. L-**Glycerol** 3-phosphate was further treated with 4-aminoantipyrine and ADOS in the presence of peroxidase to form a product for spectrophotometric detn. Av. free **glycerol** in serum sample from outpatients was 7.0 mg/mL and that in serum samples from inpatients was 8.3 mg/dL. Of 37 outpatients tested, no correlation between **triglyceride** values and **glycerol** values was obsd. Time required for total removal of free **glycerol** was 3-4 min. Bilirubin and **glycerol** almost had no effect on the detn. of **triglycerides**. The method pos. correlated with the GPO method ( $\gamma = 0.998$ ).

Trans sent 5/12/03

=> d bib ab ind 18

L8 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS  
AN 101:3552 CA  
TI Enzymic-spectrophotometric analysis of body fluids.  
PA Toyobo Co., Ltd., Japan  
SO Jpn. Kokai Tokkyo Koho, 6 pp.  
CODEN: JKXXAF

DT Patent  
LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 59011197	A2	19840120	JP 1983-113870	19830623 <--
PRAI	JP 1983-113870		19830623		

AB Body fluid constituents (including enzymes) are detd. by measuring the H<sub>2</sub>O<sub>2</sub> formed from oxidn. of the product of the body fluid constituent or enzyme with oxidative enzymes. For detn. of a body fluid constituent by the method, a body fluid sample is first reacted with a reagent mixt. contg. oxidative enzyme, peroxidase, and phenol derivs. I (R<sub>1</sub> = halogen; R<sub>2</sub> = alkyl, acyl, alkyl ether, alkoxycarbonyl, or sulfonate) (reagent-1) and then with a 2nd reagent mixt. contg. esp. a coupler agent (reagent-2). Thus, for detn. of serum triglycerides, 20 .mu.L serum samples were first incubated with 2 mL of reagent-1 soln. contg. glycerol kinase, glycerophosphate oxidase, peroxidase, and 3-acetyl-4-chlorophenol in a pH 7.0 Tris buffer at 37.degree. for 5 min and then with 1 mL of reagent-2 soln. contg. lipoprotein lipase, 4-aminoantipyrine, and N-ethyl-N-(sulfopropyl)-m-anisidine in similar Tris buffer at 37.degree. for 10 min, and the resultant color complex was measured at 540 nm to quantitate triglycerides in the sample.

IC C12Q001-28; G01N033-50

CC 9-2 (Biochemical Methods)

Section cross-reference(s): 7

ST body fluid analysis enzyme spectrophotometry; triglyceride detn serum

IT Body fluid

(anal. of, enzymic-spectrophotometric)

IT Glycerides, analysis

RL: ANT (Analyte); ANST (Analytical study)

(detn. of, in blood serum, enzymic-spectrophotometric)

IT Enzymes

RL: ANT (Analyte); ANST (Analytical study)

(detn. of, in body fluid, spectrophotometric)

IT Blood analysis

(triglycerides detn. in, enzymic-spectrophotometric)

IT 83-07-8 9003-99-0 9004-02-8 9030-66-4 9046-28-0 58020-38-5  
88795-34-0

RL: ANST (Analytical study)

(reagent contg., for triglycerides detn. in blood serum)

KR 97701789	A	19970412	WO 95JP641	A	19950403	
			WO 95JP641	A	19950403	17
			KR 96704881	A	19960904	
US 5773304	A	19980630	WO 95JP641	A	19950403	199833
			US 96704681	A	19960919	
EP 753583	A4	19971210	EP 95913411	A	19950403	199840
JP 2799835	B2	19980921	JP 9513607	A	19950131	199843
AU 696681	B	19980917	AU 9520852	A	19950403	199849
MX 9604514	A1	19971201	MX 964514	A	19960930	199936
EP 753583	B1	20001018	EP 95913411	A	19950403	200053
			WO 95JP641	A	19950403	
DE 69519160	E	20001123	DE 619160	A	19950403	200101
			EP 95913411	A	19950403	
			WO 95JP641	A	19950403	
CN 1145096	A	19970312	CN 95192343	A	19950403	200103
TW 400385	A	20000801	TW 95103384	A	19950408	200109
ES 2153030	T3	20010216	EP 95913411	A	19950403	200114

Priority Applications (No Type Date): JP 9513607 A 19950131

Cited Patents: DE 880511; EP 265933; JP 6242110; JP 63126498; No-Citns.

#### Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9623902	A1	J	11	C12Q-001/60	
Designated States (National): AU CA CN KR MX US					
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE					
JP 8201393	A		4	G01N-033/92	
AU 9520852	A			C12Q-001/60	Based on patent WO 9623902
EP 753583	A1 E		6	C12Q-001/60	Based on patent WO 9623902
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
KR 97701789	A			C12Q-001/60	Based on patent WO 9623902
US 5773304	A			G01N-033/533	Based on patent WO 9623902
EP 753583	A4			C12Q-001/60	
JP 2799835	B2		4	C12Q-001/60	Previous Publ. patent JP 8201393
AU 696681	B			C12Q-001/60	Previous Publ. patent AU 9520852
					Based on patent WO 9623902
MX 9604514	A1			C12Q-001/60	
EP 753583	B1 E			C12Q-001/60	Based on patent WO 9623902
Designated States (Regional): AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
DE 69519160	E			C12Q-001/60	Based on patent EP 753583
					Based on patent WO 9623902
CN 1145096	A			C12Q-001/60	
TW 400385	A			C12Q-001/60	
ES 2153030	T3			C12Q-001/60	Based on patent EP 753853

#### Abstract (Basic): WO 9623902 A

Quantitative analysis of cholesterol contained in high density lipoproteins (HDL) comprises: (i) adding (a) a substance which forms a complex with lipoprotein (LP) other than HDL, to a LP-contg. specimen, and (b) a surfactant; and (ii) enzymatically determining the amt. of cholesterol.

The reagent comprises a polyanion or a divalent metal ion. The cholesterol is cholesterol stearate or oxalate.

USE - The process is used in clinical and automatic analysis.

ADVANTAGE - The process is simple and highly efficient and does not require pretreatment such as centrifugal sepn.

Dwg. 0/0

Title Terms: QUALITATIVE; ANALYSE; CHOLESTEROL; HIGH; DENSITY; LIPOPROTEIN;

ADD; COMPLEX; FORMING; SUBSTANCE; SURFACTANT; ENZYME; MEASURE; AMOUNT

Derwent Class: B04; D16

International Patent Class (Main): C12Q-001/60; G01N-033/533; G01N-033/92

International Patent Class (Additional): C12Q-001/26; C12Q-001/44;

G01N-033/53

File Segment: CPI

Manual Codes (CPI/A-N): B01-D02; B04-L03A; B04-L03B; B04-L05A; B04-N05;

B05-B02A3; B07-D08; B10-B04A; B11-C08E3; B12-K04A; D05-A02C; D05-H09

=> d bib ab 1-71

L21 ANSWER 1 OF 71 CA COPYRIGHT 2003 ACS  
AN 133:293185 CA  
TI Method for quantitating **triglycerides** in very low-density  
lipoproteins and intermediate density lipoproteins  
IN Okada, Masahiko  
PA Japan  
SO PCT Int. Appl., 84 pp.  
CODEN: PIXXD2  
DT Patent  
LA Japanese  
FAN.CNT 1

*find US  
CAG*

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000060112	A1	2000/10/12	WO 2000-JP2114	20000331
	W: JP, US				
PRAI	JP 1999-128994	A	1999/04/01		
	WO 1999-JP6723	W	1999/12/01		

*NPA*

AB A method is described for selectively quantitating **triglycerides** contained in very low-d. lipoproteins (VLDL) and/or intermediate d. lipoproteins (IDL) by treating a sample with enzymes catalyzing a series of reactions leading to the formation of hydrogen peroxide or a reduced form of coenzyme from **triglyceride** in the presence of a selective reaction-accelerating agent (e.g., surfactant, polyoxyalkylene, polysaccharide), and measuring the hydrogen peroxide or the reduced form of coenzyme formed. A reagent contg. (i) a selective reaction-accelerating agent, and (ii) enzymes catalyzing a series of reactions leading to the formation of hydrogen peroxide or a reduced form of coenzyme from **triglycerides**, is also described for selectively quantitating **triglyceride** contained in VLDL and/or IDL in a sample.

RE.CNT 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 2 OF 71 CA COPYRIGHT 2003 ACS  
AN 133:132105 CA  
TI A method for quantitating **triglyceride** in specific lipoprotein  
IN Miyauchi, Kazuhito; Takada, Shizuyo; Murakami, Tomomi; Miike, Akira  
PA Kyowa Medex Co., Ltd., Japan  
SO PCT Int. Appl., 26 pp.  
CODEN: PIXXD2

*in stam*

DT Patent  
LA Japanese  
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000043537	A1	2000/07/27	WO 2000-JP246	20000120
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2360679	AA	20000727	CA 2000-2360679	20000120
	EP 1148142	A1	20011024	EP 2000-900833	20000120
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI	JP 1999-12434	A	19990120		
	WO 2000-JP246	W	20000120		

AB A convenient method is provided for quantitating **triglyceride** (TG) in a specific lipoprotein (e.g., HDL, LDL) among various lipoproteins. The method is characterized by eliminating free **glycerol** from a sample contg. free **glycerol** and TG in the specific lipoprotein, treating the residue with lipoprotein **lipase** and an enzymic system which generates hydrogen peroxide from free **glycerol**, and then, quantitating the formed hydrogen peroxide. The detn. of TG in LDL contributes to the prevention of arteriosclerosis through obtaining an index for the prodn. of small, dense LDL.

RE.CNT 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 3 OF 71 CA COPYRIGHT 2003 ACS

AN 131:41829 CA

TI Determination of LDL-**triglycerides** from blood using selective solubilization with cyclodextran and triblock copolymer

IN Wieland, Heinrich; Nauck, Matthias

PA Germany

SO PCT Int. Appl., 29 pp.

CODEN: PIXXD2

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9931512	A1	19990624	WO 1998-EP8253	19981216
	W: JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	DE 19756255	A1	19990701	DE 1997-19756255	19971217
	DE 19756255	C2	19991111		
	EP 1040354	A1	20001004	EP 1998-966630	19981216
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 2002508519	T2	20020319	JP 2000-539358	19981216
PRAI	DE 1997-19756255	A	19971217		
	WO 1998-EP8253	W	19981216		

AB The invention concerns a homogeneous assay for measuring low-d. lipoprotein **triglycerides** from blood by cyclodextrin pptn. and triblock polymer solubilization, followed by enzymic treatment and spectrophotometric **glycerol** detn. Serum is treated with .alpha.-cyclodextrin or sulfate derivs. in the presence of two-valent metal ions; LDL-**triglycerides** are selectively solubilized with polyoxyethylene-polyoxypropylene blockpolymer. **Triglycerides** are cleaved with **lipase** or esterase; **glycerol** is detd. in a spectrophotometric enzyme reaction with **glycerol kinase** and **glycerol**-3-phosphate dehydrogenase; sensitivity enhancing substances, e.g. triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase can be added. Mol. wt. of the triblock polymer is 1000-8000; the triblock polymer has a compn. A-B-A, where A represents polyoxyethylene, B represents polyoxypropylene; the mol. mass ratio of B is 75-95%. The invention also concerns a diagnostic kit contg. the necessary components; it can be used for the diagnosis of vascular diseases, esp. detecting coronary cardiac disease.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 4 OF 71 CA COPYRIGHT 2003 ACS

AN 129:65047 CA

TI Quantitative detection method of **triglycerides** in serum lipoproteins and serum-free **glycerol** by high-performance liquid chromatography

AU Okazaki, Mitsuyo; Komoriya, Norikazu; Tomoike, Hitonobu; Inoue, Noriko;

*a-type*

USUI, Shinichi; ITOH, Sekiko; HOSAKI, Seijin  
 CS College of Liberal Arts and Sciences, Laboratory of Chemistry, Tokyo  
 Medical and Dental University, Ichikawa, 272, Japan  
 SO Journal of Chromatography, B: Biomedical Sciences and Applications (1998),  
 709(2), 179-187  
 CODEN: JCBEP; ISSN: 0378-4347  
 PB Elsevier Science B.V.  
 DT Journal  
 LA English

AB We have developed a simple and reliable method for quant. detection of **triglycerides** (TG) in serum lipoproteins and serum-free **glycerol** (FG) by high-performance liq. chromatog. (HPLC). After sepn. of serum constituents using a new gel-permeation column (TSK gel Lipopropak XL, Tosoh) and a new eluent (TSK eluent LP-2, Tosoh), TG and FG were detected by online reaction using a modified reagent which contained **glycerol kinase**, **glycerol-3-phosphate oxidase** and **lipoprotein lipase**. HPLC patterns showed five peaks corresponding to chylomicrons, very-low-d., low-d., high-d. lipoproteins and FG. Abs. concns. of TG in each lipoprotein fraction and serum FG were calcd. from the corresponding peak areas using std. FG as a calibrator. Due to its very high sensitivity of peak detection, this method has become desirable for the analyses of lipoproteins of very low concns. such as in cell culture systems. This technique will contribute to a better understanding of lipoprotein TG and serum FG distribution in human and nonhuman subjects.

RE.CNT 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD  
 ALL CITATIONS AVAILABLE IN THE RE FORMAT

L21 ANSWER 5 OF 71 CA COPYRIGHT 2003 ACS

AN 126:327762 CA

TI Electrochemical method for the determination of **triglycerides**

IN Laurinavicius, Valdas-Stanislovas; Cicenienė, Regina; Kurtinaitienė, Bogumila; Meskys, Rolandas; Marcinkeviciene, Liucija; Bachmatova, Irina  
 PA Biochemijos Institutas, Lithuania

SO Lith., 12 pp.

CODEN: LIXXFS

DT Patent

LA Lithuanian

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	LT 3876	B	19960425	LT 1993-1743	19931230
PRAI	LT 1993-1743		19931230		

AB An electrochem. method for detg. **triglycerides** is disclosed which involves the use of immobilized enzymes. **Triglycerides** are hydrolyzed with **lipase** to **glycerol** and fatty acids. The **glycerol** reacts with ATP in the presence of **glycerokinase** and **MgCl<sub>2</sub>** to produce 1- $\alpha$ -glycerophosphate and ADP. The **glycerophosphate** is oxidized with **glycerophosphate oxidase** in the presence of **O<sub>2</sub>** to produce dihydroacetone phosphate plus hydrogen peroxide, which decomps. at an electrode to yield water, oxygen, and 2 electrons per peroxide mol.

L21 ANSWER 6 OF 71 CA COPYRIGHT 2003 ACS

AN 124:197530 CA

TI Determination of **triglycerides**, **glycerol**, and ATP by a polyezyme biosensor

AU Laurinavichyus, V. A.; Tsitsenė, R. A.; Kurtinaitienė, B. S.; Meshkis, R. Yu.; Martinkė, L. Yu.; Bakhmatova, I. V.

CS Inst. Biochem. Mokslininku, Vilnius, Lithuania

SO Journal of Analytical Chemistry (Translation of Zhurnal Analiticheskoi Khimii) (1995), 50(12), 1207-11

CODEN: JACTE2; ISSN: 1061-9348

PB MAIK Nauka/Interperiodica

DT Journal  
 LA English  
 AB An anal. amperometric polyenzyme system for detg. **triglycerides** and products of their metab. was developed. This system dets. the **triglyceride** concn. in blood plasma over a physiol. concn. range with an accuracy sufficient for medical diagnostic purposes. **Triglyceride** hydrolysis was performed with sol. **lipase**, and **glycerol** phosphorylation and oxidn. were carried out with immobilized glycerokinase and glycerophosphate **oxidase**. The anal. time was 2 to 3 min; the useful life of the enzyme membrane was one week. The anal. system had a linear dependence of the electrode current on the concns. of **triglycerides**, **glycerol**, glycerophosphate, and ATP to 1.5, 0.3, 0.6, and 0.8 mM, resp.

L21 ANSWER 7 OF 71 CA COPYRIGHT 2003 ACS

AN 121:132488 CA

TI Development and practical evaluation of an amperometric **triglyceride** sensor

AU Feldbruegge, R.; Renneberg, R.; Spener, F.

CS Institute of Chemical and Biochemical Sensor Research, Wilhelm-Klemm-Strasse 8, Munster, D-48149, Germany

SO Sensors and Actuators, B: Chemical (1994), 19(1-3), 365-7  
 CODEN: SABCEB; ISSN: 0925-4005

DT Journal

LA English

AB An amperometric **triglyceride** sensor with an enzymic hydrolysis unit and a sensor unit with a thin polyurethane membrane contg. **glycerol kinase** (EC 2.7.1.30) and **glycerol** -3-phosphate **oxidase** (EC 1.1.3.21), sandwiched between 2 dialysis membranes and mounted onto a platinum electrode in a flow-through cell combined with pumps, autosampler and potentiostat was developed. The complete system contains a computer-controlled data acquisition and analyzing unit. Complete hydrolysis of **triglyceride** samples is attained within 15 min with the aid of a previously freeze-dried mix contg. triacylglycerol acylhydrolase (**lipase** EC 3.1.1.3), carboxylic-ester hydrolase (esterase EC 3.1.1.1), detergent, ATP and buffer. With a linear sensor signal from 5 to 1000 .mu.mol/L, 25 samples per h can be analyzed. The std. deviation was 2%.

L21 ANSWER 8 OF 71 CA COPYRIGHT 2003 ACS

AN 119:112965 CA

TI Two-component liquid-stable **triglyceride** reagent system

IN Wegfahrt, Paul F., Jr.; Bolstad, Ralph E.; Modrovich, Ivan E.

PA USA

SO U.S., 4 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5221615	A	19930622	US 1985-794177	19851114
PRAI	US 1985-794177		19851114		

AB A stable **triglyceride** assay soln. for use with a chromogen system is comprised of a soln. buffered with a Zwitterionic buffering agent and/or TRIS and stabilized with sorbitol, gelatin, and ammonium sulfate. Soln. pH is 6.8-8.0. The functional enzymes are **lipase**, **glycerol kinase**, and **glycerol** phosphate **oxidase**. The preferred chromogen system is based on the inclusion of 4-aminoantipyrine for use in combination with **oxidase** to yield a detectable chromogen compd. A preferred **triglyceride** enzyme soln. contains MOPS, TRIS, K<sub>4</sub>Fe(CN)<sub>6</sub>, **lipase**, **glycerol kinase**, **glycerol** phosphate **oxidase**, ammonium sulfate, and gelatin. A preferred chromogen

soln. contains gelatin, MOPS, TRIS, ATP, MgCl<sub>2</sub>, Na<sub>2</sub>EDTA, 4-aminoantipyrine, p-chlorophenol, Triton X-100, and, peroxidase. The net mixed working soln. was stable .apprx.2 wk at 2-8.degree.; the sep. solns. were stable .gtoreq.18 mo at 2-8.degree..

L21 ANSWER 9 OF 71 CA COPYRIGHT 2003 ACS

AN 118:120122 CA

TI Discordant results for determinations of **triglycerides** in pig sera

AU Tuten, Tom; Robinson, Keith A.; Sgoutas, Demetrios S.

CS Sch. Med., Emory Univ., Atlanta, GA, 30322, USA

SO Clinical Chemistry (Washington, DC, United States) (1993), 39(1), 125-8  
CODEN: CLCHAU; ISSN: 0009-9147

DT Journal

LA English

AB **Triglyceride** concns. in pig sera were recently detd. by 3 fully enzymic methods (Kodak Ektachem 700, Hitachi 707, and Abbott EPx) and significantly lower values were obtained than those obtained with chem. or enzymic methods based on chem. hydrolysis. All methods used involve microbial **lipases** for liberating **glycerol** from **glycerides** and **glycerol** phosphate dehydrogenases or **oxidases** for subsequent oxidn. The methods were validated against ref. methods by using fresh human sera and survey materials. The discordant results were not from matrix sample-method interaction but from incomplete hydrolysis of pig serum **triglycerides** by the lipolytic enzymes. When serum **triglycerides** from 10 pigs showing the highest biases were hydrolyzed by microbial **lipases** and the reaction mixt. was subjected to TLC and gas chromatog., the predominant end products were palmitoyl monoglyceride and a mixt. of free fatty acids with the following compn. (fatty acid as percent of total .+- SD): 16:0, 7.8 .+- .2; 18:0, 5.4 .+- .2.2; 18:1, 53 .+- .12; 18:2, 31 .+- .4.6; and 18:3, 2.5 .+- .1. Assuming that the **lipases** exhibit the usual specificity toward the 1 and 3 positions of the **triglyceride**, the data suggest that, in pig, **triglycerides** 18:1 and 18:2 occupy the 1 and 3 positions and 16:0 (palmitic acid) predominantly occupies the 2 position. **Triglycerides** of this structure may not be well hydrolyzed by the typical lipolytic enzymes in clin. assays.

L21 ANSWER 10 OF 71 CA COPYRIGHT 2003 ACS

AN 118:97292 CA

TI A simple enzymic quantitative analysis of **triglycerides** in tissues

AU Danno, Hiroshi; Jincho, Yuu; Budiyo, Slamet; Furukawa, Yuji; Kimura, Shuichi

CS Fac. Agric., Tohoku Univ., Sendai, 981, Japan

SO Journal of Nutritional Science and Vitaminology (1992), 38(5), 517-21  
CODEN: JNSVA5; ISSN: 0301-4800

DT Journal

LA English

AB A method to measure the **triglyceride** levels in tissues by using a modified enzymic kit is reported. This enzymic kit was originally designed to be used to measure the **triglyceride** levels in plasma. The method of **triglyceride** level detn. includes dissolving the tissue lipid exts. in an alc. Before using the enzymic kit directly, the lipids were dissolved in tert-Bu alc., then a Triton X-100/MeOH mixt. was added (1:1). The presence of org. surfactants such as tert-Bu alc. and MeOH and of a surfactant such as Triton X-100 did not interfere with the enzymic activity. This method enabled **triglyceride** levels to be detd. between 10 and 90 nmol, using a spectrophotometer to measure the absorbances.

L21 ANSWER 11 OF 71 CA COPYRIGHT 2003 ACS

AN 114:77763 CA



TI Spleen **lipase** determination with **triglycerides** as substrate

IN Totsu, Yoshifumi; Shirahase, Yasushi

PA International Reagents Corp., Japan

SO Jpn. Kokai Tokkyo Koho, 3 pp.

CODEN: JKXXAF

DT Patent

LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 02186999	A2	19900723	JP 1989-5699	19890111
	JP 2894710	B2	19990524		
PRAI	JP 1989-5699		19890111		

AB Spleen **lipase** (I) is detd. in a reaction mixt. contg. **triglyceride**, diglyceride **lipase** reactive to mono- and diglycerides but not with **triglycerides**, and **glycerol** detn. reagents. The method is simple and highly sensitive, and it is useful in diagnosis of spleen disorders. Detn. of I in 30 human blood serum samples was shown; the result was comparable to that of previous known turbidity method.

L21 ANSWER 12 OF 71 CA COPYRIGHT 2003 ACS

AN 113:168455 CA

TI An enzymic ultramicromethod for measuring the blood serum **triglycerides**

AU Xiao, Zhongyi

CS Peop. Rep. China

SO Laboratornoe Delo (1990), (3), 44-6

CODEN: LABDAZ; ISSN: 0023-6748

DT Journal

LA Russian

AB The enzymic method for detg. serum **triglycerides** is based on their hydrolysis by lipoprotein **lipase** to **glycerol** and fatty acid. Mg ions activate **glycerol kinase** which with **glycerol** and ATP form glycerol-3-phosphate (GP). GP, under the influence of glycerophosphatoxidase, is converted to phosphodihydroxyacetone and H<sub>2</sub>O<sub>2</sub>. The latter under peroxidase is degraded which is estd. using the chromogenic agents p-chlorobenzophenol and 4-amidoantipyrine. The reproducibility of the method is good and only 6 .mu.L of serum is required.

L21 ANSWER 13 OF 71 CA COPYRIGHT 2003 ACS

AN 113:148374 CA

TI Method and reagent containing **lipases** for enzymic determination of **triglycerides**

IN Imamura, Shigeyuki; Takahashi, Mamoru; Misaki, Hideo; Matsuura, Kazuo

PA Toyo Jozo Co., Ltd., Japan

SO Ger. Offen., 20 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 3912226	A1	19891109	DE 1989-3912226	19890413
	DE 3912226	C2	19960509		
	JP 01265899	A2	19891023	JP 1988-92912	19880415
	JP 2647684	B2	19970827		
	FR 2630129	A1	19891020	FR 1989-4903	19890413
	FR 2630129	B1	19940713		
	US 5126246	A	19920630	US 1989-435003	19891109
PRAI	JP 1988-92912		19880415		
	US 1989-328457		19890324		

AB A reagent for detn. of serum **triglycerides** contains **lipase(s)** in combination with monoglyceride **lipase (I)** from *Bacillus stearothermophilus* H-165 and known reagents for detn. of **glycerol**. I is highly active and allows rapid detn. of **triglycerides**. Phys. and biochem. properties of I are given. Thus, a reagent for spectrochem. detn. of **triglycerides** contained 100 mM PIPES buffer (pH 7.3), 5 mM ATP, 2 mM MgCl<sub>2</sub>, 0.7 units glycerokinase/mL, 5.0 units glycerophosphate **oxidase**/mL, 5.0 units peroxidase/mL, 0.2% Triton X-100, 233 units **lipase**/mL, 0.15 unit I/mL, 0.03% 4-aminoantipyrine, and 0.03% 3,5-dimethoxy-N-ethyl-N-(2-hydroxy-3-sulfopropyl)aniline. This reagent (3.0 mL) was mixed with 20 .mu.M serum and incubated at 37.degree.; the endpoint of the reaction (detd. from absorbance at 600 nm) was reached within 2 min.

L21 ANSWER 14 OF 71 CA COPYRIGHT 2003 ACS

AN 112:231896 CA

TI Thermostable lipoprotein **lipase** of thermophilic actinomycetes: production, purification, characterization and use in blood **triglyceride** determination

IN Takeda, Akira; Kamei, Tomoko; Kageyama, Masao; Motosugi, Kenzo

PA Unitika Ltd., Japan

SO Eur. Pat. Appl., 12 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 354551	A2	19900214	EP 1989-114726	19890809
	EP 354551	A3	19910529		
	EP 354551	B1	19951108		
	R: DE, FR, GB, IT, NL				
	JP 02049581	A2	19900219	JP 1988-199639	19880809
	US 5173417	A	19921222	US 1989-391017	19890809
	US 5244798	A	19930914	US 1992-929743	19920817
	US 5232846	A	19930803	US 1992-929744	19920903
PRAI	JP 1988-199639		19880809		
	US 1989-391017		19890809		

AB Thermostable lipoprotein **lipase (I)** is obtained by cultivating a thermophilic actinomycete, particularly *Streptomyces* 7825, and purifn. of the enzyme from the culture medium by std. methods. The purified enzyme is characterized and utilized for blood **triglyceride** detn. Thus, *Streptomyces* 7825 was cultured in a medium contg. peptone (5%), KH<sub>2</sub>PO<sub>4</sub> (0.1%), Na<sub>2</sub>HPO<sub>4</sub> (0.1%), MgSO<sub>4</sub> (0.05%), yeast ext. (0.03%), olive oil (0.2%), and Tween 40 (0.5%). After 3 days of culturing with aeration and stirring at 50.degree., cells were removed by filtration and I was purified from the culture medium chromatog. on phenyl-Sepharose and Sephadex G-75. Purified I, which gave a single band on SDS-PAGE, had a specific activity of 1.2 U/mg, a pH optimum of 8-9, and a temp. optimum of .apprx.60.degree.. I retained essentially 100% of its activity when heated to 60.degree. for 15 min at pH 4-7. I was used in the colorimetric detn. of blood **triglycerides**. The detn. involved assaying **glycerol**, formed by the reaction of I with **triglycerides**, with a coupled enzyme system consisting of **glycerol kinase**, **glycerol 3-phosphate oxidase**, and peroxidase.

L21 ANSWER 15 OF 71 CA COPYRIGHT 2003 ACS

AN 112:174992 CA

TI Falsely low estimation of **triglycerides** in lipemic plasma by the enzymic **triglyceride** method with modified Trinder's chromogen

AU Shepherd, Mark D. S.; Whiting, Malcolm J.

CS Dep. Biochem. Chem. Pathol., Flinders Med. Cent., Bedford Park, 5042, Australia

SO Clinical Chemistry (Washington, DC, United States) (1990), 36(2), 325-9  
 CODEN: CLCHAU; ISSN: 0009-9147  
 DT Journal  
 LA English  
 AB The enzymic assay of **triglyceride**, based on the use of L-**glycerol-3-phosphate oxidase** (EC 1.1.3.21) and a modified Trinder's chromogen involving 4-chlorophenol, is subject to strong neg. interference at concns. of **triglyceride** >20 mmol/L, such as occur in grossly lipemic plasma. This interference is caused by the rapid utilization of oxygen, resulting in the reaction becoming transiently anaerobic. The dye product already formed may then be reduced (bleached) by acting as an alternative electron acceptor for **glycerol-3-phosphate oxidase**. Redn. of the dye leads to a marked decrease in final absorbance at 505 nm. Grossly underestimated values for **triglyceride** concns., apparently within the linear range of the assay, may therefore be inadvertently obtained with equil. methods. Samples giving unexpectedly low results for lipemic plasma should be reassayed after diln. or with use of a smaller vol. of sample.

L21 ANSWER 16 OF 71 CA COPYRIGHT 2003 ACS  
 AN 112:95060 CA  
 TI Simultaneous assay for cholesterol and **triglycerides**  
 IN Bates, Diane Marian; Alejos, Michael A.  
 PA Abbott Laboratories, USA  
 SO PCT Int. Appl., 21 pp.  
 CODEN: PIXXD2

DT Patent  
 LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8902925	A1	19890406	WO 1988-US3169	19880920
	W: AU, JP, KR, US				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	AU 8824863	A1	19890418	AU 1988-24863	19880920
	EP 395654	A1	19901107	EP 1988-908629	19880920
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	JP 01108998	A2	19890426	JP 1988-237453	19880921
	CA 1323554	A1	19931026	CA 1988-578065	19880921
	AU 9222808	A1	19921119	AU 1992-22808	19920907
PRAI	US 1987-99890		19870922		
	WO 1988-US3169		19880920		

AB A method for the simultaneous detn. of cholesterol and **triglycerides** uses a single reagent system. The reagent system is reacted with the specimen such that each of the substrates reacts with their resp. reactant simultaneously. The change in absorbance or fluorescence of the resulting reaction mixt. is measured at a plurality of wavelengths which are characteristic for each of the substrates to be detd. The amt. of cholesterol and **triglyceride** can be detd. by either an endpoint or reaction rate measurement. The reagent system comprises an enzyme having cholesterol esterase activity, a chromogenic oxygen acceptor, microperoxidase, and cholesterol **oxidase** for detn. of cholesterol; and **lipase**, ATP, PEP (phosphoenolpyruvate), **glycerol kinase**, pyruvate **kinase**, LDH (lactate dehydrogenase) and NAD(P)H or analogs thereof for detn. of **triglycerides**. Thus, a reagent system contg. Na cholate, 4-aminoantipyrine, PhOH, **lipase**, cholesterol **oxidase**, microperoxidase, NADH, PEP, ATP, MgSO<sub>4</sub>, Tris buffer, succinic acid, pyruvate **kinase**, **glycerol kinase**, and LDH was mixed with sample at a ratio of 101:1, resp., and after 3 min absorbance was read at 340 (**triglyceride**) and 500 nm (cholesterol). Concns. were detn. by comparison to std. curves.

L21 ANSWER 17 OF 71 CA COPYRIGHT 2003 ACS

AN 109:186423 CA  
 TI Applications of serum **triglyceride** to automatic analyzer  
 (discrete type, continuous-flow type)  
 AU Nobuoka, Manabu; Matsumiya, Kazuto  
 CS Clin. Lab., Asahikawa Med. Coll. Hosp., Japan  
 SO Medical Technology (Tokyo, Japan) (1988), 16(5), 409-17  
 CODEN: METCDS; ISSN: 0389-1887  
 DT Journal; General Review  
 LA Japanese  
 AB A review and discussion with 15 refs. on the anal. of serum  
**triglycerides** (TGs) by discrete- and continuous-flow-type  
 automated analyzer systems, based on enzymic assay which uses lipoprotein  
**lipase** to hydrolyze the TGs and subsequent detn. of products, esp.  
**glycerol**, by **glycerol kinase**, glycerophosphate  
**oxidase-peroxidase**, or **glycerol oxidase**  
 -peroxidase. The app., detectors, coenzymes, reagents, and problems in  
 relation to the 2 automated analyzer systems for TGs are also discussed.

L21 ANSWER 18 OF 71 CA COPYRIGHT 2003 ACS

AN 109:19872 CA  
 TI Enzymic determination of **triglycerides** in body fluids  
 IN Akiba, Tetsunori; Suzuki, Satoshi; Matsunaga, Kuniyoshi  
 PA Amano Pharmaceutical Co., Ltd., Japan  
 SO Jpn. Kokai Tokkyo Koho, 8 pp.  
 CODEN: JKXXAF  
 DT Patent  
 LA Japanese  
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 62240000	A2	19871020	JP 1986-82992	19860410
	JP 06051000	B4	19940706		
	US 4999289	A	19910312	US 1987-34452	19870406
PRAI	JP 1986-82991		19860410		
	JP 1986-82992		19860410		

AB **Triglycerides** in a biol. sample are measured with **lipase**  
 in the presence of a surfactant that practically does not inhibit the  
 enzyme activity at a concn. of .ltoreq.5%. The **lipase** converts  
**triglycerides** to **glycerol** and fatty acids at a ratio of  
 >5%. The surfactant promotes the conversion. A serum sample or std. was  
 treated with a reagent contg. **glycerol kinase**,  
 .alpha.-glycerophosphate **oxidase**, peroxidase, ATP,  
 4-aminoantipyrine, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine, MgCl<sub>2</sub>,  
**lipase**, and Triton X-100 (<5.0%) at 37.degree. for 10 min, and the  
 absorbance at 555 nm was measured for **triglyceride** detn.

L21 ANSWER 19 OF 71 CA COPYRIGHT 2003 ACS

AN 108:127836 CA  
 TI Determination of serum **triglyceride** by an enzymic method  
 AU Li, Jianzhai; Wang, Shu  
 CS Dep. Biochem., Natl. Inst. Geriatr., Beijing, Peop. Rep. China  
 SO Zhonghua Yixue Jianyan Zazhi (1987), 10(5), 262-5  
 CODEN: CHCCDO; ISSN: 0253-973X  
 DT Journal  
 LA Chinese  
 AB Blood serum was treated with a reagent mixt. and incubated at 37.degree.  
 for 10 or 15 min. Absorbance was measured at 500 nm for detn. of  
**triglyceride** concn. The reagent mixt. was a Tris-HCl buffer (pH  
 7.6) contg. lipoprotein **lipase**, glycerokinase, ATP,  
**glycerol** phosphate **oxidase**, peroxidase, Na cholate,  
 MgSO<sub>4</sub>, 4-aminoantipyrine, 4-chlorophenol, and Triton X 100. The std. plot  
 was linear up to 22.6 mmol/L. This method is simple, rapid, sensitive,  
 and precise.

- L21 ANSWER 20 OF 71 CA COPYRIGHT 2003 ACS  
 AN 107:171719 CA  
 TI A method for the sequential colorimetric determination of serum **triglycerides** and cholesterol  
 AU Sharma, Ajit; Artiss, Joseph D.; Zak, Bennie  
 CS Sch. Med., Wayne State Univ., Detroit, MI, 48201, USA  
 SO Clinical Biochemistry (1987), 20(3), 167-72  
 CODEN: CLBIAS; ISSN: 0009-9120  
 DT Journal  
 LA English  
 AB A simple enzymic-spectrophotometric method for the sequential detn. of **triglycerides** and cholesterol from a single serum sample was developed. In this 2-stage procedure, the **triglycerides** and cholesterol esters are 1st hydrolyzed to **glycerol** and free cholesterol, resp., with simultaneous scavenging of the liberated free fatty acids, a technique that ensures clarity of the sample. The **glycerol** is subsequently reacted to result in an intense red chromogen with a peak absorption max. at 510 nm following a series of enzymic reactions. In the 2nd stage, addn. of cholesterol **oxidase** leads to oxidn. of free cholesterol generated from the cholesterol esters in the 1st stage and the free cholesterol normally present in the sample, yielding in a similar fashion the identical red chromogen whose absorbance is also measured at 510 nm. Results obtained with the proposed method demonstrate good correlation with established individual procedures for **triglycerides** and cholesterol.
- L21 ANSWER 21 OF 71 CA COPYRIGHT 2003 ACS  
 AN 106:152321 CA  
 TI New enzymic system (GlyDH-DHAK method) for the assay of **triglycerides**  
 AU Tsubota, Hiroyuki  
 CS Ogata Inst. Med. Chem. Res., Tokyo, Japan  
 SO Kenkyu Hokoku - Ogata Igaku Kagaku Kenkyusho (1985) 21-9  
 CODEN: OIKHDE; ISSN: 0285-4554  
 DT Journal  
 LA Japanese  
 AB A new enzymic assay system is described for detn. of blood **triglycerides** (TG), which uses dihydroxyacetone **kinase** (DHAK) in addn. to lipoprotein **lipase**, **glycerol** dehydrogenase (GlyDH), and NAD. The addn. of DHAK markedly stimulated the reaction of the GlyDH mediated conversion of **glycerol** and NAD to dihydroxyacetone and NADH, resp.; the NADH formed was measured at 340 nm to obtain the quantity of TG. The **glycerol** (.ltoreq.2000 mg/dL) reaction was completed within 3 min in the new enzymic system, and showed assay linearity in **glycerol** concn. of .ltoreq.2000 mg/dL. The percent recovery of the new enzymic system for **glycerol** and TG (triolein) was .apprx.150%. Thus, the new enzymic system is useful for accurate assay of blood TG.
- L21 ANSWER 22 OF 71 CA COPYRIGHT 2003 ACS  
 AN 105:149047 CA  
 TI A fluorometric method for the determination of **triglycerides** in nanomolar quantities  
 AU Mendez, Armando J.; Cabeza, Carmen; Hsia, S. L.  
 CS Sch. Med., Univ. Miami, Miami, FL, 33101, USA  
 SO Analytical Biochemistry (1986), 156(2), 386-9  
 CODEN: ANBCA2; ISSN: 0003-2697  
 DT Journal  
 LA English  
 AB A fluorometric assay for **triglycerides** in nanomole quantities is described. **Glycerol** is liberated from **triglycerides** with **lipase** from *Chromobacterium viscosum*, then converted by **glycerol kinase** to **glycerol 3-phosphate**, which is oxidized by **glycerol 3-phosphate oxidase**, producing

H2O2. The H2O2 ultimately forms a peroxidase-catalyzed fluorogen with p-hydroxyphenylacetic acid. The excitation and emission wavelengths of the fluorogen are 325 and 415 nm, resp. The assay is linear for 0.05-35 nmol **triglycerides** with triolein as std. The method would be esp. useful for measuring **triglycerides** in liq. chromatog. fractions of lipoproteins.

L21 ANSWER 23 OF 71 CA COPYRIGHT 2003 ACS

AN 105:57200 CA

TI Fluorometric procedures for measuring **triglyceride** concentrations in small amounts of tissue and plasma

AU Nemeth, P. M.; Hitchins, O. E.; Solanki, L.; Cole, T. G.

CS Sch. Med., Washington Univ., St. Louis, MO, 63110, USA

SO Journal of Lipid Research (1986), 27(4), 447-52

CODEN: JLPRAW; ISSN: 0022-2275

DT Journal

LA English

AB **Triglycerides** can be specifically hydrolyzed by **lipase** from *Rhizopus arrhizus* in the presence of hog liver esterase and SDS. The **glycerol** produced can then be measured by sequential reactions with glycerokinase, pyruvate **kinase**, and lactate dehydrogenase: **glycerol** and ATP are converted to **glycerol** 3-phosphate and ADP by glycerokinase; the ADP reacts with phosphoenolpyruvate and pyruvate **kinase** to yield pyruvate; the pyruvate is converted to lactate with lactate dehydrogenase, and the cofactor NAD is simultaneously reduced to NADH. Either the disappearance of NADH or the appearance of NAD was detd. fluorometrically, with 10- to 100-fold greater sensitivity than by spectrophotometry. In addn., enzymic cycling of NAD was used to increase the sensitivity of the assay over 1000-fold and thereby provided accurate measurement of <1 ng of **triglyceride**. Results obtained from the 3 fluorometric methods were highly correlated with an automated periodate oxidn. method using serum samples and lipid exts. of muscle tissue.

L21 ANSWER 24 OF 71 CA COPYRIGHT 2003 ACS

AN 103:101140 CA

TI Hydroxyurea interferes negatively with **triglyceride** measurement by a **glycerol oxidase** method

AU McPherson, Richard A.; Brown, Kevin D.; Agarwal, Raghunath P.; Threatte, Gregory A.; Jacobson, Robert J.

CS Med. Cent., Georgetown Univ., Washington, DC, 20007, USA

SO Clinical Chemistry (Washington, DC, United States) (1985), 31(8), 1355-7

CODEN: CLCHAU; ISSN: 0009-9147

DT Journal

LA English

AB The neg. interference of hydroxyurea was examd. in the detn. of **triglycerides** in serum of patients receiving orally Hydrea (500-mg capsules hydroxyurea) for the treatment of myeloproliferative diseases by using an enzymic cascade assay with lipoprotein **lipase**, **glycerol oxidase**, and peroxidase in the Technicon RA-1000 analyzer. Hydroxyurea added to serum samples appeared to inhibit the action of **glycerol oxidase**, with a stoichiometric relation to the concn. of substrate (a decrease of roughly 2.4 mmol/L in measured **triglyceride** per 1 mmol of hydroxyurea/L). Similarly, increasing concns. of hydroxyurea added to a **glycerol** soln. (as substrate) decreased the measured concns. of **triglycerides**. Hydroxyurea did not inhibit the peroxidase reaction but interferes with **glycerol oxidase**. Hydroxyurea had no effect on the detn. of **triglycerides** in the Astra-8 system which uses **glycerol kinase**. This neg. interference by hydroxyurea could complicate proper assessment of a patients nutritional status during long-term administration of hydroxyurea.

L21 ANSWER 25 OF 71 CA COPYRIGHT 2003 ACS

L21 ANSWER 35 OF 71 CA COPYRIGHT 2003 ACS  
 AN 97:212064 CA  
 TI Colorimetric **triglyceride** determination  
 PA Wako Pure Chemical Industries, Ltd., Japan  
 SO Jpn. Kokai Tokkyo Koho, 7 pp.  
 CODEN: JKXXAF

DT Patent  
 LA Japanese  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 57137858	A2	19820825	JP 1981-23745	19810220
PRAI	JP 1981-23745		19810220		

AB For **triglycerides** detn., free **glycerol** and reducing substances present in a biol. sample are completely decompd. with HIO<sub>4</sub> (0.0005-0.003M) at pH 0-3.5, and then **triglycerides** in the sample are enzymically hydrolyzed into **glycerol** and fatty acids. The **glycerol** formed quant. is detd. to est. the **triglyceride** content in the sample. Thus, 20 .mu.L serum and 0.6 mL HIO<sub>4</sub>-2H<sub>2</sub>O (0.04 g/100 mL) were mixed, incubated at 37.degree. for 3 min, and to this was added 2.5 mL soln. contg. lipoprotein **lipase** 3500, **glycerokinase** 200, **glycerol** 3-phosphate **oxidase** 120, peroxidase 200 units, Na ATP 100, 4-aminoantipyrine 9, (p-chlorophenol) 70 mg, Mg(OAc)<sub>2</sub> 4 mM, Triton X405 20 mg in 100 mL 0.05M Tris buffer. The mixt. was incubated at 37.degree. for 10 min and the color developed was measured at 505 nm.

L21 ANSWER 36 OF 71 CA COPYRIGHT 2003 ACS  
 AN 97:195247 CA  
 TI Serum **triglycerides** determined colorimetrically with an enzyme that produces hydrogen peroxide  
 AU Fossati, Piero; Prencipe, Lorenzo  
 CS Ames Res. Dev. Lab., Miles Italiana S.p.A., Cavenago Brianza, 20040, Italy  
 SO Clinical Chemistry (Washington, DC, United States) (1982), 28(10), 2077-80  
 CODEN: CLCHAU; ISSN: 0009-9147

DT Journal  
 LA English

AB Serum **triglycerides** are hydrolyzed by **lipase**, and the released **glycerol** is assayed in a reaction catalyzed by **glycerol kinase** and L-.alpha.-glycerolphosphate **oxidase** in a system that generates H<sub>2</sub>O<sub>2</sub>. The H<sub>2</sub>O<sub>2</sub> is monitored in the presence of horseradish peroxidase with 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone as the chromogenic system. The high absorbance of this chromogen system at 510 nm affords useful results with a sample/reagent vol. ratio as low as 1:150, and a blank sample measurement is not needed. A single, stable working reagent is used; the reaction is complete in 15 min at room temp. The std. curve is linear for **triglyceride** concns. as great as 13.6 nmol/L. Av. anal. recovery of **triglycerides** in human serums is 100.1%, and within-run and between-run precision studies showed relative std. deviations of .ltoreq.1.6 and .ltoreq.3.0%, resp. The method is suitable for automation.

L21 ANSWER 37 OF 71 CA COPYRIGHT 2003 ACS  
 AN 97:123194 CA  
 TI A simple, fully enzymic bioluminescent assay for **triglycerides** in serum  
 AU Bjoerkhem, I.; Sandelin, Kerstin; Thore, Anders  
 CS Karolinska Inst., Huddinge Univ. Hosp., Huddinge, S-141 86, Swed.  
 SO Clinical Chemistry (Washington, DC, United States) (1982), 28(8), 1742-4  
 CODEN: CLCHAU; ISSN: 0009-9147

DT Journal  
 LA English

AB In this fully enzymic bioluminescent assay, **triglycerides** are cleaved by **lipase** (EC 3.1.1.3) and carboxylesterase (EC 3.1.1.1), and the **glycerol** obtained is phosphorylated with ATP and **glycerol kinase** (EC 2.7.1.30). The ATP consumed in the latter reaction is detd. by the firefly luciferin-luciferase reaction, and corresponds to the concn. of **triglycerides**. All the enzymic reactions and the bioluminescent reading can be performed in the same test tube. The precision of the assay varied between 1 and 7% at different concns. of **triglycerides**. There was a good agreement with a fully enzymic method based on spectrophotometry ( $r = 0.98$ ).

L21 ANSWER 38 OF 71 CA COPYRIGHT 2003 ACS

AN 97:106555 CA

TI Microcalorimetric determination of serum **triglycerides** and cholesterol

AU Das, Y. T.; Chattopadhyay, S. K.; Brown, H. D.

CS New Jersey Agric. Exp. Stn., Rutgers, State Univ., New Brunswick, NJ, 08903, USA

SO Analytical Letters (1982), 15(B6), 519-27

CODEN: ANALBP; ISSN: 0003-2719

DT Journal

LA English

AB Microcalorimetrically measured reaction heats between various amts. of human serum and 700 IU **lipase** (EC 3.1.1.3) and between serum and 8 units cholesterol **oxidase** (EC 1.1.3.6) were linearly related to the contents of **triglycerides** ( $r = 1.00$ ) and cholesterol ( $r = 0.99$ ), resp. However, when sera from human volunteers were subjected to a comparative study between the microcalorimetric and spectrophotometric methods, the correlation coeffs. were ( $r = 0.70$  and  $r = 0.71$ ), presumably due to individual variations in free **glycerol** and cholesterol ester constituents that were deliberately omitted in the microcalorimetric method but were routinely included in the std. clin. (multienzymic) spectrophotometric method. The contribution of interfering substances, if any, is not totally ruled out.

L21 ANSWER 39 OF 71 CA COPYRIGHT 2003 ACS

AN 97:51953 CA

TI Elimination of free **glycerol** in serum **triglyceride** enzymic determination

AU Asayama, Hitoshi; Komikado, Yoshitake; Kurano, Hiroshi

CS Dep. Clin. Lab., Natl. Nara Hosp., Nara, Japan

SO Eisei Kensa (1982), 31(4), 705-8

CODEN: EIKEAS; ISSN: 0367-052X

DT Journal

LA Japanese

AB Detn. of serum **triglycerides** (TG) in the presence of free **glycerol** (FG) is based on the reaction of FG with ATP in the presence of **glycerol kinase** to form ADP and L-.alpha.-glycerophosphate, and the latter compd. is oxidized by glycerophosphate **oxidase** to produce dihydroxyacetone phosphate and H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> produced is reacted with ESPT (undefined), in the presence of peroxidase (POD), to form a colorless quinone deriv. for the elimination of FG. Sep., the TG are treated with lipoprotein **lipase** to give FG, which is treated similarly as above, but the H<sub>2</sub>O<sub>2</sub> formed is treated with ESPT and 4-aminoantipyrine, in the presence of POD, to produce a quinone deriv. with max. absorbance at 550 nm. Only 20 .mu.L of blood serum is needed. The color reaction reaches equil. .apprx.8 min after elimination of FG. The calibration curve is linear to 1000 mg/dL. Hb at concns. of <500 mg/dL has no effect on the anal., whereas bilirubin and ascorbic acid at concns. of 10-20 mg/dL affect the results slightly.

L21 ANSWER 40 OF 71 CA COPYRIGHT 2003 ACS

AN 96:177450 CA



TI Analysis of **triglycerides**  
 IN Leon, Luis; Ahmad, Syed I.; Yeh, Chien Kuo  
 PA Technicon Instruments Corp., USA  
 SO Eur. Pat. Appl., 16 pp.  
 CODEN: EPXXDW  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 44615	A2	19820127	EP 1981-302661	19810615
	EP 44615	A3	19820203		
	EP 44615	B1	19840530		
	R: BE, CH, DE, FR, GB, IT, NL, SE				
	US 4338395	A	19820706	US 1980-171112	19800721
	CA 1144051	A1	19830405	CA 1981-374028	19810327
	AU 8169457	A1	19820128	AU 1981-69457	19810413
	AU 539052	B2	19840906		
	JP 57155998	A2	19820927	JP 1981-62672	19810427
PRAI	US 1980-171112		19800721		

AB In the title serum glyceride enzymic detn. with **lipase** and a **glycerol** detn. reagent mixt., interference from endogenous **glycerol** and pyruvate is removed enzymically. In 1 example, human serum is treated with **glycerol kinase**, ATP, MnCl<sub>2</sub>, pyruvate **kinase**, phosphoenolpyruvate, CaCl<sub>2</sub>, glutamate-pyruvate transaminase, glutamate, and Tris buffer to remove **glycerol** and pyruvate. Next, EDTA (for **kinase** inactivation), 5-polyoxyethylene lauric amide, and Tris buffer were added. Glycerides were then converted to **glycerol** by **lipase**, the soln. dialyzed, and the dialyzed **glycerol** combined with NADH, MgSO<sub>4</sub>, and KCl. The resulting soln. was passed through an enzyme coil with immobilized **glycerol kinase**, pyruvate **kinase**, and lactate dehydrogenase. The decrease in absorbance was then monitored. Modifications of this example are also described.

L21 ANSWER 41 OF 71 CA COPYRIGHT 2003 ACS

AN 96:100533 CA

TI Enzymic assay and reagent for **glycerol** and **triglycerides**

IN Lauderdale, Vivian R.

PA Beckman Instruments, Inc., USA

SO U.S., 12 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 4309502	A	19820105	US 1980-164720	19800630
PRAI	US 1980-164720		19800630		

AB A reagent kit for the simultaneous detn. of **glycerol** and **triglycerides** in a sample (blood serum) without the need for a sep. **glycerol** assay contains a **lipase**, **glycerol kinase**, ATP, .gtoreq.1 reagent for assay of **glycerol** 1-phosphate or ADP, and a surfactant for activating the **lipase**. In the absence of the surfactant, the Staphylococcus epidermidis **lipase** does not cleave **triglycerides** to **glycerol**. The anal. can be made in a single cuvette at either 340 or 520 nm. Thus, 1 mL reagent contg. 50 IU/L S. epidermidis **lipase**, 0.3 mM ATP, 0.3 mM reduced coenzyme, 5 mM Mg<sup>2+</sup>, 0.37 mM phosphoenolpyruvate, 2900 IU/L pyruvate **kinase**, and 1485 IU/L lactic dehydrogenase was incubated at 37.degree. for 5 min with 10 .mu.L of a reagent contg. 250 IU/mL **glycerol kinase** in a cuvette in a spectrometer set at 340 nm, and the absorbance was measured. A blood serum sample, 10 .mu.L, was added and incubated for 5 min, and the

absorbance was measured again. To the cuvette was added the surfactant reagent (7.5% each of Na dihexyl sulfosuccinate and p-tert-octylphenoxypolyethoxyethanol, and the absorbance was measured again after a 10-min incubation. **Glycerol** is detd. from the difference between the 1st and 2nd reading and **triglycerides** by the difference between the 2nd and 3rd; formulas are given.

L21 ANSWER 42 OF 71 CA COPYRIGHT 2003 ACS

AN 95:76516 CA

TI Method and reagent for determining **triglycerides** in biological material

IN Klose, Sigmar; Roeder, Albert; Schneider, Walter

PA Boehringer Mannheim G.m.b.H. , Fed. Rep. Ger.

SO Ger. Offen., 19 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2950381	A1	19810619	DE 1979-2950381	19791214
	CA 1148456	A1	19830621	CA 1980-365792	19801128
	US 4368261	A	19830111	US 1980-213702	19801205
	EP 30718	A1	19810624	EP 1980-107835	19801211
	EP 30718	B1	19821201		
	R: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	AT 1913	E	19821215	AT 1980-107835	19801211
	JP 56092798	A2	19810727	JP 1980-174800	19801212
	JP 58005677	B4	19830201		
	DD 155211	C	19820519	DD 1980-226023	19801212
PRAI	DE 1979-2950381		19791214		
	EP 1980-107835		19801211		

AB A method for enzymic detn. of **triglycerides** is described which uses **lipase**, esterase, **glycerol kinase** from *Bacillus stearothermophilus*, and activators. The resulting **glycerol** 1-phosphate or ADP are detd. by known methods. **Glycerol kinase** from *B. stearothermophilus* is used because this enzyme is not inactivated by **lipase** activators. The assay can be performed in soln. or with test strips. In 1 example, **triglycerides** were detd. with a reagent contg. 20 mM phosphate buffer (pH 7), 4 mM MgSO<sub>4</sub>, 0.35 mM SDS (as activator), 0.2 mM NADH, 0.44 mM ATP, 0.36 mM PEP, 6 units/mL lactate dehydrogenase, 1 unit/mL pyruvate kinase, 80 units/mL **lipase**, 0.6 unit/mL esterase, and 1 unit/mL **glycerol kinase** (omitted in blank). Other activators and other systems for **glycerol** 1-phosphate and ADP detns. are described.

L21 ANSWER 43 OF 71 CA COPYRIGHT 2003 ACS

AN 95:57650 CA

TI An improved **triglycerides** assay for biological fluids and reagents therefor

IN Denney, Jerry William

PA American Monitor Corp., USA

SO Brit. UK Pat. Appl., 9 pp.

CODEN: BAXXDU

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	GB 2050601	A	19810107	GB 1979-19558	19790605
	GB 2050601	B2	19830525		
PRAI	GB 1979-19558		19790605		

AB In a **triglyceride** assay and reagent system in which

**glycerol** from **lipase**-hydrolyzed **triglycerides** is coupled to a NAD/NADH indicator system,  $\text{Fe}^{3+}$  is introduced into the initial reaction mixt., the NADH formed reduces  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , and the  $\text{Fe}^{2+}$  is reacted with a chelating agent which forms a chromophore for measurement, thereby improving the sensitivity and performance of the assay. Thus, to a buffered mixt. of the Fe chelator 9-(2-pyridyl)acenaphtho[1,2-e]-as-triazine sulfonate, ATP,  $\text{MgSO}_4$ , **lipase**, **glycerol** 3-phosphate dehydrogenase, and **glycerol kinase**, a sample of serum to be tested was added, followed by phenazine methosulfate, NAD, and  $\text{Fe}^{3+}$ . This mixt. was incubated at 37.degree. for 20 min and the absorbance detd. at 610 nm. Comparison with the absorbance developed for solns. of known **triglyceride** compn. gave the **triglyceride** content of the serum. A linear response was obsd. up to .apprx.500 mg/dL of **triglyceride**.

L21 ANSWER 44 OF 71 CA COPYRIGHT 2003 ACS  
 AN 94:117379 CA  
 TI Composition for the quantification of **glycerol** ATP and **triglycerides**  
 IN Esders, Theodore W.; Goodhue, Charles T.  
 PA Eastman Kodak Co., USA  
 SO U.S., 14 pp. Cont.-in-part of U.S. Ser. No. 715,797, abandoned.  
 CODEN: USXXAM  
 DT Patent  
 LA English  
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 4241178	A	19801223	US 1978-867641	19780106
	CA 1100023	A1	19810428	CA 1977-282354	19770708
	JP 53024892	A2	19780308	JP 1977-99418	19770819
	JP 60012040	B4	19850329		
	FR 2362395	A1	19780317	FR 1977-25353	19770819
	FR 2362395	B1	19840224		
	GB 1590736	A	19810610	GB 1977-34974	19770819
	JP 57026600	A2	19820212	JP 1981-72130	19810513
	JP 01031880	B4	19890628		
	JP 61293397	A2	19861224	JP 1986-12270	19860124
	JP 02046200	B4	19901015		
PRAI	US 1976-715797		19760819		

AB Methods and compns. are described for the detn. of **glycerol**, ATP, **triglycerides**, **glycerol kinase**, **lipase**, and .alpha.-glycerophosphate in aq. solns. As an example, serum **triglycerides** were detd. by hydrolyzing the **triglycerides** to **glycerol** with **lipase**, converting the **glycerol** to L-.alpha.-glycerophosphate with **glycerol kinase** and ATP, oxidizing glycerophosphate with .alpha.-glycerophosphate **oxidase** in the presence of O to produce  $\text{H}_2\text{O}_2$ , then detg. the amt. of  $\text{H}_2\text{O}_2$ .  $\text{H}_2\text{O}_2$  is detd. with peroxidase and a chromogen, such as 4-aminoantipyrine. The .alpha.-glycerophosphate **oxidase** method produced comparable results as previously published methods, and the relative std. deviations were 5.1 and 2.6% for normal and abnormal serums, resp. Electron acceptors other than O were compared.

L21 ANSWER 45 OF 71 CA COPYRIGHT 2003 ACS  
 AN 94:117138 CA  
 TI Ultramicro determination of serum **triglycerides** by bioluminescent assay  
 AU Werner, Mario; Gabrielson, Dale G.; Eastman, John  
 CS Med. Cent., George Washington Univ., Washington, DC, 20037, USA  
 SO Clinical Chemistry (Washington, DC, United States) (1981), 27(2), 268-71  
 CODEN: CLCHAU; ISSN: 0009-9147  
 DT Journal

LA English  
 AB The described ultramicro (1-.mu.L samples) assay of serum of plasma **triglycerides** is potentially applicable to 1-nL samples and to isolated cells. This method involves only 3 reactions: (a) enzymic hydrolysis with **lipase** and .alpha.-chymotrypsin; (b) conversion by **glycerol kinase** of the liberated **glycerol** and of ATP added in excess to **glycerol** 1-phosphate and to ADP; and (c) assay of the residual ATP, by the luciferin-luciferase reaction. The assay was optimized with respect to **glycerol kinase**, buffer, pH, temp., and ATP. Performance characteristics compare well with those of traditional **triglyceride** assays.

L21 ANSWER 46 OF 71 CA COPYRIGHT 2003 ACS

AN 94:79901 CA

TI Test device, composition and method for the determination of **triglycerides**

IN Gupta, Surendra Kumar; Chaudhari, Panna Ramjibhai

PA Miles Laboratories, Inc., USA

SO Eur. Pat. Appl., 26 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 19253	A1	19801126	EP 1980-102614	19800512
	EP 19253	B1	19820512		
	R: DE, FR, GB, IT, SE				
	US 4259440	A	19810331	US 1979-40559	19790521
	CA 1143637	A1	19830329	CA 1980-349848	19800414
	JP 55156599	A2	19801205	JP 1980-62920	19800514
	JP 56046799	B4	19811105		
	AU 8058565	A1	19801127	AU 1980-58565	19800520
	AU 517714	B2	19810820		
PRAI	US 1979-40559		19790521		

AB **Triglycerides** are detd. with a mixt. of **lipase**, cholesterol esterase, and a **glycerol** assay system. In 1 example, serum **triglycerides** were hydrolyzed by a mixt. of cholesterol esterase and **lipase** ( ratio of 0.88:10 units) to fatty acids and **glycerol**. **Glycerol** was detd. with an assay system contg. phosphoenolpyruvate, ATP, pyruvate **kinase**, lactate dehydrogenase, NADH, and MgSO<sub>4</sub>. The NADH produced was detd. spectrometrically at 340 nm. **Triglyceride** detns. with other **glycerol** assay systems are also described.

L21 ANSWER 47 OF 71 CA COPYRIGHT 2003 ACS

AN 93:145754 CA

TI A fully automatic, enzymic simultaneous method for the determination of total cholesterol and **triglycerides** in blood serum or blood plasma

AU Christ, G. A.; Iversen, J. S.; Simane, Z.; Harders, H. D.

CS Darmstadt, 6100, Fed. Rep. Ger.

SO AutoAnal. Innovationen: Problemlösungen Med., Forsch. Ind., Dok. Vortr. Technicon Symp., 7th (1979), Meeting Date 1978, Volume 2, 182-8.

Editor(s): Hoef, Sibylle. Publisher: Tech. GmbH, Bad Vilbel, Fed. Rep. Ger.

CODEN: 43XIAA

DT Conference

LA German

AB Cholesterol esters were hydrolyzed by using **lipase** from *Candida cylindracea*. The total cholesterol then was detd. by reaction with cholesterol **oxidase**, followed by reaction with 4-aminopyrazolone-5, PhOH, and peroxidase and measurement at 505 nm. **Triglycerides** were also hydrolyzed with the **lipase**. The

resultant **glycerol** was detd. after reaction with ATP and glycerokinase, followed by reaction with **glycerol** 3-phosphate dehydrogenase and hydrazine and fluorometric measurement of the NADH at 460 nm. A 2-channel AutoAnalyzer was used with a sample capacity of 40 samples/h. When compared with the Liebermann-Burchard method for cholesterol, and a manual alk.-EtOH extn. for **triglycerides**, this completely automated, simultaneous method had regression coeffs. (r) of 0.99.

L21 ANSWER 48 OF 71 CA COPYRIGHT 2003 ACS

AN 93:110211 CA

TI Test composition for the determination of **triglycerides** and its use

IN Nagai, Toshiaki; Terada, Osamu; Uwajima, Takayuki; Mihara, Akira; Aisaka, Kazuo; Akita, Hiroko; Shimizu, Yoshiaki

PA Kyowa Hakko Kogyo Co., Ltd., Japan

SO Eur. Pat. Appl., 16 pp.

CODEN: EPXXDW

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 10296	A1	19800430	EP 1979-104011	19791017
	EP 10296	B1	19821201		
	R: DE, FR, GB, IT				
	JP 55055263	A2	19800423	JP 1978-127328	19781018
	JP 58050719	B4	19831111		
PRAI	JP 1978-127328		19781018		

AB **Triglycerides** are detd. by a method which comprised of 3 systems; (1) a system converting **triglycerides** to **glycerol** and fatty acids, (2) a system with **glycerol** oxidase oxidizing **glycerol** to glyceraldehyde + H<sub>2</sub>O<sub>2</sub> with uptake of O<sub>2</sub>, and (3) a system for detg. glutaraldehyde or H<sub>2</sub>O<sub>2</sub> or the uptake of O<sub>2</sub>. Thus, a reagent contg. **lipase**, .alpha.-chymotrypsin, and 4-aminoantipyrine and a reagent contg. glucose oxidase and peroxidase in pH 2.0 buffer were dissolved in a soln. of phenol and Triton X-100 in pH 8.0 TES buffer to make the test soln. An 0.2 mL blood serum sample was added to 3 mL test soln. The mixt. was held at 37.degree. for 10 min and the development of color was read at 505 nm. Absorbance was proportional to **triglyceride** content.

L21 ANSWER 49 OF 71 CA COPYRIGHT 2003 ACS

AN 93:40772 CA

TI Fluorometric and colorimetric enzymic determination of **triglycerides** (triacylglycerols) in serum

AU Winartasaputra, Handani; Mallet, Victorin N.; Kuan, Shia S.; Guilbault, George G.

CS Dep. Chem., Univ. New Orleans, New Orleans, LA, 70122, USA

SO Clinical Chemistry (Washington, DC, United States) (1980), 26(5), 613-17  
CODEN: CLCHAU; ISSN: 0009-9147

DT Journal

LA English

AB A colorimetric and a fluorometric enzymic method for the detn. of **triglycerides** in serum are described. Samples are incubated with microbial **lipase** for 10 min, and the **glycerol** released from the **triglycerides** is oxidized by NAD<sup>+</sup> in the presence of **glycerol** dehydrogenase. In the fluorometric method, the resulting NADH is in turn oxidized by resazurin as catalyzed by diaphorase to form resorufin, a highly fluorescent compd. In the colorimetric method, the NADH is oxidized by coupling with a tetrazolium salt/diaphorase system to form formazan, a highly colored compd. Calibration curves, constructed by plotting change in fluorescence or absorbance vs. concn. of **triglycerides**, were linear to 6 and 5 g **triglycerides**/L

serum for the fluorometric and colorimetric methods, resp. The assays require only 5 and 15 .mu.L of serum for fluorometry and colorimetry, resp. The time for anal. for either method is <15 min. The results correlate well with those obtained by the Dow Diagnostic Kit method, a colorimetric method in which **glycerol kinase** and **glycerol 1-phosphate dehydrogenase** form NADH from ATP and NAD+ in the presence of **glycerol** and **glycerol 1-phosphate**.

L21 ANSWER 50 OF 71 CA COPYRIGHT 2003 ACS  
 AN 92:142744 CA  
 TI Enzymic kinetic determination of serum **triglycerides**  
 AU Bartl, K.; Neumann, U.; Ziegenhorn, J.  
 CS Biochemica Werk Tutzing, Boehringer Mannheim G.m.b.H., Tutzing, D-8132, Fed. Rep. Ger.  
 SO Laboratoriumsmedizin (1979), 6(3), 120-2  
 CODEN: LABOD3; ISSN: 0342-3026  
 DT Journal  
 LA German  
 AB A 1-stage assay for the kinetic detn. of serum **triglycerides** by using **lipase/esterase/glycerol kinase** /pyruvate **kinase** (ATP)/lactate dehydrogenase is described. The method was adapted to the ENI GEMSAEC Fast Analyzer, the Union Carbide Centrifichem System 400, and the Abbott ABA-100 Analyzer. It yielded satisfactory results with regard to precision, linearity, and accuracy. The reagents used are also suited for the detn. of **triglycerides** on the LKB Reaction Rate Analyzer 8600 or 2086, and on the Vitatron Akes in a 2-stage assay (starting with **glycerol kinase**).

L21 ANSWER 51 OF 71 CA COPYRIGHT 2003 ACS  
 AN 91:136657 CA  
 TI Determination of **triglycerides**  
 IN Denney, Jerry William  
 PA American Monitor Corp., USA  
 SO Ger. Offen., 36 pp.  
 CODEN: GWXXBX  
 DT Patent  
 LA German  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2847202	A1	19790613	DE 1978-2847202	19781030
	US 4245041	A	19810113	US 1977-858187	19771207
	JP 54080192	A2	19790626	JP 1978-96051	19780807
	CA 1125151	A1	19820608	CA 1978-312452	19781002
	FR 2411411	A1	19790706	FR 1978-32374	19781116
	FR 2411411	B1	19840706		
	BE 872547	A1	19790606	BE 1978-2057466	19781206
PRAI	US 1977-858187		19771207		

AB **Triglycerides** are detd. by measuring the oxidn. state of Fe after enzymic treatment in an NAD/NADH system. **Triglycerides** were hydrolyzed by **lipase** and converted to **glycerol 1-phosphate** by ATP and **glycerol kinase**. **Glycerol-1-phosphate** was converted to dihydroxyacetone phosphate by **glycerol phosphate dehydrogenase** with simultaneous redn. of NAD to NADH which reduced Fe3+ to Fe2+ which was bound by an iron chelator forming an intense chromophore. Measurement of the chromophore quantifies the amt. of **triglyceride** present in biol. fluids.

L21 ANSWER 52 OF 71 CA COPYRIGHT 2003 ACS  
 AN 91:136564 CA  
 TI Critical study of methods of the enzymic determination of serum **triglycerides**  
 AU Ekindjian, O. G.; Duchassaing, D.  
 CS Lab. Biochim., Hop. A. Chenevriier, Creteil, Fr.

SO Annales de Biologie Clinique (1979), 37(3), 175-80  
 CODEN: ABCLAI; ISSN: 0003-3898  
 DT Journal  
 LA French  
 AB A crit. study is presented of 2 enzymic methods for detn. of serum **triglycerides** which differ mainly in the choice of hydrolytic agents: esterase and hydrolase by the Roche method; **lipase** and esterase in the Boehringer method. The estn. of the resulting free **glycerol** requires 3 sequential enzymic reactions (**glycerol kinase**, pyruvate **kinase**, lactate dehydrogenase ending in the measurement of the consumed NADH. A study of the kinetics of the methods in 2 stages was carried out using std. solns. of **glycerol** and human serums of variable concn. Optimal conditions for reaction time and temp. were found. Trials of repeatability and reproducibility from day to day were carried out together with an evaluation of precision.

L21 ANSWER 53 OF 71 CA COPYRIGHT 2003 ACS  
 AN 90:117444 CA  
 TI Simultaneous, in-flux continuous determination of serum **triglycerides** and cholesterol by enzymic method  
 AU Tallet, F.; Caillens, H.; Raichvarg, D.  
 CS Serv. Biochim., Hop. Cochin, Paris, Fr.  
 SO Pharmaciens Biologiste (Paris) (1978), 12(117), 529-31  
 CODEN: PHBIA7; ISSN: 0553-9323  
 DT Journal  
 LA French  
 AB Serum **triglycerides** and cholesterol were detd. simultaneously with the Technicon Autoanalyzer II. Total cholesterol was detd. after incubation with cholesterol esterase and oxidn. by cholesterol **oxidase** by measuring the intensity of color of 4-aminophenazone oxide in the presence of phenol at 505 nm, the oxide being formed by the action of H2O2 in the presence of peroxidase. **Triglycerides** were detd. by releasing **glycerol** with **lipase** esterase, which then became involved in a chain of enzymic transformations to produce NAD which was measured photometrically at 340 nm. The standardization curve was linear, and the height of the peaks recorded must be multiplied by 0.04 to obtain the concn. in grams/L. The repeatability was good, and contamination was negligible.

L21 ANSWER 54 OF 71 CA COPYRIGHT 2003 ACS  
 AN 88:185810 CA  
 TI Multilayered analytical elements for the determination of **triglycerides** and glycerin  
 IN Esders, Theodore Walter; Goodhue, Charles Thomas; Dappen, Glen Marshall; Warburton, Charles Donald  
 PA Eastman Kodak Co., USA  
 SO Ger. Offen., 52 pp.  
 CODEN: GWXXBX

DT Patent  
 LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2737286	A1	19780223	DE 1977-2737286	19770818
	DE 2737286	C2	19850502		
	CA 1114269	A1	19811215	CA 1977-284983	19770818
	JP 53024893	A2	19780308	JP 1977-99417	19770819
	JP 60017520	B4	19850502		
	FR 2362396	A1	19780317	FR 1977-25355	19770819
	FR 2362396	B1	19831209		
	GB 1590738	A	19810610	GB 1977-34976	19770819
	JP 57163495	A2	19821007	JP 1981-184559	19811119
PRAI	US 1976-715796		19760819		
AB	A multilayer anal. system for <b>triglyceride</b> or glycerin detn. in				

liq. samples is described, in which glycerin, released by **lipase** action, is converted to L-.alpha.-glycerophosphate by glycerin **kinase** and glycerophosphate then is oxidized by glycerophosphate **oxidase** with the simultaneous redn. of either an electron acceptor dye or O. Thus, a poly(ethyleneterephthalate) film support layer was coated with a reagent layer contg. deionized gelatin, 4-isopropoxy-1-naphthol, 5,5-dimethyl-1,3-cyclohexadione, peroxidase, ATP, .alpha.-glycerophosphate **oxidase**, and glycerokinase. The H2O2 formed upon glycerophosphate oxidn. reacts with peroxidase and 4-isopropoxy-1-naphthol to give a detectable product. A layer of poly(isopropylacrylamide) is applied and upon this, a diffusion-reflection layer of TiO2, cellulose acetate, **lipase**, and octylphenoxypolyethoxyethanol (surfactant activator of **lipase**) is formed. The **triglyceride** content in blood serum was estd. by applying the sample to the multilayer system and after 8 min at 37.degree., measuring the reflection d. at 660 nm.

L21 ANSWER 55 OF 71 CA COPYRIGHT 2003 ACS

AN 88:148555 CA

TI Comparison of three automated methods of serum **triglyceride** analysis

AU Postle, A. D.; Goodland, F. C.

CS Fac. Med., Gen. Hosp., Southampton, UK

SO Annals of Clinical Biochemistry (1978), 15(1), 18-24

CODEN: ACBOBU; ISSN: 0004-5632

DT Journal

LA English

AB Two new automated methods for serum **triglyceride** anal. are described and compared with each other and with an established chem. method. The new methods are a chem. method requiring no **glycerol** blank correction and a com. available fully enzymic method requiring no prior lipid extn. The chem. method involved extn. with nonane-propan-2-ol, reaction with Na methoxide to form Me fatty acids, and finally, after conversion of the **glycerol** to H2CO, reaction with acetylacetone to form 3,5-diacetyl-1,4-dihydrolutidine and detn. of the absorbance at 410 nm. The enzymic method involved reaction with **lipase**, esterase, glycerokinase, pyruvate **kinase**, and lactate dehydrogenase, (com. reagents and substrates). The decrease in fluorescence then was detd. at 470 nm. Both methods had higher quality control precision and faster sampling rates than the established method. Comparison of duplicate analyses of a random series of serum samples by all 3 methods gave a closer correlation between the new methods than between either and the conventional method.

L21 ANSWER 56 OF 71 CA COPYRIGHT 2003 ACS

AN 88:101159 CA

TI Automated procedure for kinetic measurement of total **triglycerides** (as **glycerol**) in serum with the Gilford System 3500

AU Lehnus, Gary; Smith, Lynn

CS Gilford Instrum. Lab., Inc., Oberlin, OH, USA

SO Clinical Chemistry (Washington, DC, United States) (1978), 24(1), 27-31

CODEN: CLCHAU; ISSN: 0009-9147

DT Journal

LA English

AB An automated procedure was used on the Gilford 3500 Computer-Directed Analyzer to measure serum **triglycerides** indirectly by using aq. **glycerol** stds. Most enzymic methods require long hydrolysis or awkward sapon. The method of G. Bucolo and H. David (1973), in which **lipase** and **glycerol kinase** are used, was modified. The described kinetic procedure eliminates the need for a serum blank. It uses Eskalab bulk reagents and reduces both time and cost per test by measuring the decreasing NADH concn. from the **glycerol kinase** reaction at 340 nm after enzymic hydrolysis at room temp. The change in absorbance of the std. during a 14-s measuring time was used



in the ratiometric calcn. of the unknowns. A stable aq. std. is used that can be reliably and accurately prepd. Reagent blank drift did not affect the results. A correlation coeff. of 0.991 for comparison with the manual endpoint method and a typical relative std. deviation of 2.25% show this method to be accurate and reliable.

L21 ANSWER 57 OF 71 CA COPYRIGHT 2003 ACS

AN 88:85533 CA

TI Improvement on serum **triglyceride** determination. Part 2

AU Shibata, Hidehito; Nakajima, Nobuyuki; Nonaka, Urao

CS Ogata Inst. Med. Chem. Res., Tokyo, Japan

SO Kenkyu Hokoku - Ogata Igaku Kagaku Kenkyusho (1974) 53-8

CODEN: OIKHDE; ISSN: 0285-4554

DT Journal

LA Japanese

AB During the detn. of serum **triglycerides** by the enzymic method of S. Yoshida and U. Nonaka, (1973) that uses lipoprotein **lipase**, **glycerol kinase**, and **glycerol 3-phosphate dehydrogenase**, the detn. of NADH at UV wavelengths was replaced by detg. the formation of formazan at a visible wavelength (570 nm) after the addn. of phenazine methosulfate and Nitro Blue Tetrazolium. The modified method was simpler, more specific, and faster than the original method; however, the reagents of the modified system were less stable than those of the original system.

L21 ANSWER 58 OF 71 CA COPYRIGHT 2003 ACS

AN 88:34166 CA

TI Studies on enzymes. Part CXLIV. A simple colorimetric method for determination of serum **triglycerides** with lipoprotein **lipase** and **glycerol dehydrogenase**

AU Sugiura, Mamoru; Oikawa, Tsutomu; Hirano, Kazuyuki; Maeda, Hidemi; Yoshimura, Hiroko; Sugiyama, Masayasu; Kuratsu, Taeko

CS Dep. 2nd Pharm., Tokyo Coll. Pharm., Tokyo, Japan

SO Clinica Chimica Acta (1977), 81(2), 125-30

CODEN: CCATAR; ISSN: 0009-8981

DT Journal

LA English

AB A simplified enzymic procedure to det. accurately serum **triglycerides** is described. Thus, serum **triglycerides** were hydrolyzed completely to free fatty acids and **glycerol** by lipoprotein **lipase** from *Pseudomonas fluorescens*. The released **glycerol** was oxidized with **glycerol dehydrogenase** from *Erwinia aroideae* in the presence of NAD, and the redn. of the enzyme-linked NAD was coupled to the redn. of Nitro Blue Tetrazolium as chromogen with phenazine methosulfate serving as an intermediate electron carrier of NADH. The absorbance at 570 nm was measured. The method requires only 20  $\mu$ L serum and 10-min incubation and is rapid and simple. **Triglycerides**  $\leq 1000$  mg/dL serum may be detd. There was good correlation with results obtained by the **glycerol kinase** method (correlation coeff.,  $r$ , 0.989) or the acetylacetone method ( $r$  0.979).

L21 ANSWER 59 OF 71 CA COPYRIGHT 2003 ACS

AN 87:180124 CA

TI Integral element for the detection of **glycerol** or **triglycerides**

AU Anon.

CS UK

SO Research Disclosure (1977), 161, 94-8

CODEN: RSDSBB; ISSN: 0374-4353

DT Journal

LA English

AB Elements are described for detn. of **triglycerides**/**glycerol** in liq. that require no reagent mixing and that can be

used for automated detns. The element contains **lipase** for hydrolysis of the **triglyceride**, **glycerol kinase** for conversion of **glycerol** to L-.alpha.-glycerophosphate, an **oxidase**, an electron acceptor, and an indicator for detg. the H2O2 formed. Total **glycerol** was detd. in serum samples in 5-7 min at 37.degree. by using the element, and the results were quantitated by reflectometry. These results compared favorably with the semi-automated chem. method of Kessler and Lederer.

L21 ANSWER 60 OF 71 CA COPYRIGHT 2003 ACS

AN 86:167490 CA

TI Reagent for the determination of **triglycerides**

IN Rauscher, Elli; Bernt, Erich; Gruber, Wolfgang; Determann, Helmut

PA Boehringer Mannheim G.m.b.H., Fed. Rep. Ger.

SO Ger., 4 pp. Addn. to Ger. 2,229,849.

CODEN: GWXXAW

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2535953	B1	19761202	DE 1975-2535953	19750812
	DE 2535953	C2	19770721		
	AT 7604163	A	19790615	AT 1976-4163	19760608
	AT 354406	B	19790110		
	GB 1502204	A	19780222	GB 1976-24061	19760610
	CS 219874	P	19830325	CS 1976-4473	19760706
	CS 219875	P	19830325	CS 1976-6611	19760706
	CA 1057179	A1	19790626	CA 1976-256485	19760707
	DK 7603317	A	19770213	DK 1976-3317	19760722
	DK 144856	B	19820621		
	DK 144856	C	19821108		
	SU 641884	D	19790105	SU 1976-2386217	19760730
	NL 7608735	A	19770215	NL 1976-8735	19760806
	FR 2321126	A2	19770311	FR 1976-24214	19760806
	FR 2321126	B2	19801010		
	CH 607028	A	19781130	CH 1976-10143	19760809
	BE 845042	A4	19770210	BE 1976-169690	19760810
	SE 7608919	A	19770213	SE 1976-8919	19760810
	SE 420216	B	19810921		
	SE 420216	C	19820114		
	DD 126398	W	19770713	DD 1976-194266	19760810
	HU 175208	P	19800628	HU 1976-B01627	19760811
	JP 52023110	A2	19770221	JP 1976-96608	19760812
PRAI	DE 1975-2535953		19750812		

AB The title reagent, contg. buffer, coenzyme, and enzyme solns., is prepd. for the detn. of **triglycerides** in blood serum. Thus, the following mixts. are made: (1) 0.05 M Na phosphate buffer (pH 7) contg. 4 mM MgSO4, 0.01% Na dodecyl sulfate, and 0.015% cetylstearyl alc. polyglycol ether; (2) coenzyme mixt. contg. NADH 10, ATP 22, and phosphoenolpyruvate 18 mM; (3) enzyme soln. contg. **lipase** (*Rhizopus arrhizus*) 400, carboxyl esterase (microorganism) 50, pyruvate **kinase** 50, and lactate dehydrogenase 300 Units/mL in (NH4)2SO4 suspension; and (4) **glycerol kinase** 150 Units/mL in (NH4)2SO4 suspension. In practice, 2.5 mL of mixts. 1, 2, and 3 are mixed with 0.05 mL blood serum, incubated for 10 min at 20-5.degree., and the absorbance is detd. Then 0.01 mL (4) is added, and the absorbance detd. after 10 min. The difference between values at 365, 340, or 334 nm can be used to calc. the **triglyceride** concn.

L21 ANSWER 61 OF 71 CA COPYRIGHT 2003 ACS

AN 85:139427 CA

TI Enzymic simultaneous analysis of **triglyceride** and total cholesterol with the Perkin-Elmer C4B

AU Nennstiel, H. J.; Alich, R.  
 CS Pforzheim, Fed. Rep. Ger.  
 SO Aerztliche Laboratorium (1976), 22(6), 185-9  
 CODEN: AELAAH; ISSN: 0001-9526  
 DT Journal  
 LA German  
 AB The Perkin-Elmer C4B app. simultaneously detd. cholesterol and **triglycerides** in blood serum by enzymic methods by using the cholesterol esterase-cholesterol **oxidase**-peroxidase and **lipase-glycerol kinase** -lactate dehydrogenase reactions, resp. On a day-by-day routine basis, the precisions for **triglycerides** and cholesterol were 2.8 and 3.07%, resp.

L21 ANSWER 62 OF 71 CA COPYRIGHT 2003 ACS  
 AN 83:203480 CA  
 TI Improved method for enzymic determination of serum **triglycerides**  
 AU Ziegenhorn, Joachim  
 CS Biochem. Werk Tutzing, Boehringer Mannheim G.m.b.H., Tutzing, Fed. Rep. Ger.  
 SO Clinical Chemistry (Washington, DC, United States) (1975), 21(11), 1627-9  
 CODEN: CLCHAU; ISSN: 0009-9147  
 DT Journal  
 LA English  
 AB An enzymic method for detg. serum **triglycerides** (triacylglycerols) was described. The **triglycerides** were hydrolyzed by a mixt. of **lipase** and esterase. The **glycerol** released was detd. by kinetic fixed-time anal., by using **glycerol kinase**, pyruvate **kinase**, and lactate dehydrogenase. Through addn. of the competitive inhibitor ATP, the Michaelis const. of pyruvate **kinase** apparently was increased, considerably extending the linearity of the assay. There was no need for serum blanks or reagent blanks. The method was adapted to a centrifugal analyzer (the ENI GEMSAEC). It yielded satisfactory results with regard to precision, accuracy, and insensitivity to interferences.

L21 ANSWER 63 OF 71 CA COPYRIGHT 2003 ACS  
 AN 83:175165 CA  
 TI Rapid enzymic hydrolysis of **triglycerides**  
 IN Komatsu, Stanley K.  
 PA American Hospital Supply Corp., USA  
 SO U.S., 4 pp.  
 CODEN: USXXAM  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 3898130	A	19750805	US 1974-451735	19740318
PRAI	US 1974-451735		19740318		

AB Serum **triglycerides** were detd. by measuring the released **glycerol** from its esterified form by the combined action of a pancreatic **lipase** and a microbial **lipase** in the presence of a bile salt. Serum or plasma (50 .mu.l) with **triglyceride** values of 0-500 mg% was added to a reaction mixt. contg. pancreatic **lipase** (180 **lipase** units), Candida **lipase** (80 **lipase** units). Na taurodeoxycholate (3 mg), lactate dehydrogenase (10 IU), pyruvate **kinase** (10 IU), NADH (0.75 .mu.mole), phosphoenolpyruvate (1.5 .mu.mole) di-Na ATP (0.5 .mu.mole), MgCl2 (0.0067M), and K phosphate buffer (0.1M, pH 7.0). The 3 ml reaction mixt. was incubated .apprx.5 min, 25-37.degree.. The absorbence was measured at 340 nm. Thereafter, 10 units of **glycerol kinase** was added and the mixt. was incubated for 5 min and the absorbence again measured at 340 nm; the

difference in absorbence is proportional to the **triglyceride** content.

L21 ANSWER 64 OF 71 CA COPYRIGHT 2003 ACS

AN 82:82569 CA

TI Determination of serum **triglyceride** by enzymic hydrolysis

AU Takahashi, Zyuro

CS Sch. Med., Osaka Univ., Osaka, Japan

SO Rinsho Kagaku (Nippon Rinsho Kagakkai) (1974), 2(4), 425-32

CODEN: RIKAAN; ISSN: 0370-5633

DT Journal

LA Japanese

AB **Triglycerides** were hydrolyzed to **glycerol** and fatty acids by the lipoprotein **lipase** of *Pseudomonas fluorescens*. **Glycerol** was transformed then to pyruvate in the presence of glycerokinase, pyruvate **kinase**, ATP, and phosphoenolpyruvate. After treatment with hydrazine, pyruvate was converted into its hydrazone which exhibited a max. absorbance at 450 nm, under basic conditions. The true amt. of **triglyceride** was calcd. by a formula from the absorbance value. The values obtained by this method correlated well with those detd. by chem. sepn.

L21 ANSWER 65 OF 71 CA COPYRIGHT 2003 ACS

AN 82:70061 CA

TI **Triglycerides**. Determination after enzymic saponification

AU Wahlefeld, August W.

CS Biochem. Werk Tutzing, Boehringer Mannheim G.m.b.H., Tutzing/Obb., Fed. Rep. Ger.

SO Methoden Enzym. Anal., 3. Neubearbeitete Erweiterte Aufl. (1974), Volume 2, 1878-82. Editor(s): Bergmeyer, Hans Ulrich. Publisher: Verlag Chem, Weinheim/Bergstr., Ger.

CODEN: 29GMAP

DT Conference

LA German

AB Serum **triglycerides** are hydrolyzed by **lipases**, specifically and quant., only when the substrate is in the form of an emulsion. The principle of the method is based on 3 reaction: (1) **triglyceride** hydrolysis to 1,2-diglyceride and fatty acid by **lipase** in the presence of dodecyl sulfate; (2) 1,2-diglyceride hydrolysis to 2-monoglyceride and fatty acid, catalyzed by **lipase** and esterase; and (3) 2-monoglyceride hydrolysis to **glycerol** and fatty acid, catalyzed by esterase. **Glycerol** is then detd. with ATP phosphoenolpyruvate and NADH by coupling glycerokinase, pyruvate **kinase**, and lactic dehydrogenase reactions. The decrease in NADH absorbance at 340 nm is detd. (pH 7-8, 25.degree.), and the relative std. deviation is 5%.

L21 ANSWER 66 OF 71 CA COPYRIGHT 2003 ACS

AN 81:60323 CA

TI Automated determination of serum **triglycerides** by an enzymic method

AU Dingeon, B.; Rouillet, M. J.; Rouillet, A.

CS Lab. Cent. Biochim., Hop. Jules-Courmont, Pierre-Benite, Fr.

SO Annales de Biologie Clinique (1974), 32(2), 127-34

CODEN: ABCLAI; ISSN: 0003-3898

DT Journal

LA French

AB A simple and rapid method is described for the detn. of serum **triglycerides**, by use of enzymes and an LKB 8600 reaction rate analyzer. The enzymic hydrolysis (**lipase**) is directly coupled to the detn. of **glycerol** in a one-step procedure (pyruvic **kinase** and lactic dehydrogenase).

L21 ANSWER 67 OF 71 CA COPYRIGHT 2003 ACS

AN 81:35247 CA  
 TI Method and reagents for determining **triglycerides**  
 IN Wahlefeld, August W.; Moellering, Hans; Gruber, Wolfgang; Bernt, Erich;  
 Roeschlau, Peter  
 PA Boehringer Mannheim G.m.b.H.  
 SO Ger., 5 pp.  
 CODEN: GWXXAW  
 DT Patent  
 LA German  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2229849	B1	19731220	DE 1972-2229849	19720619
	DE 2229849	C2	19740725		
	AT 324282	B	19750825	AT 1973-2164	19730312
	IT 990535	A	19750710	IT 1973-22837	19730410
	HU 167097	P	19750828	HU 1973-B01423	19730411
	NL 165845	B	19801215	NL 1973-5350	19730417
	NL 165845	C	19810515		
	DD 104367	C	19740312	DD 1973-170421	19730425
	DK 144643	B	19820426	DK 1973-2452	19730504
	DK 144643	C	19820927		
	CS 166842	P	19760329	CS 1973-3274	19730508
	US 3862009	A	19750121	US 1973-365355	19730530
	GB 1395126	A	19750521	GB 1973-27695	19730611
	CA 994658	A1	19760810	CA 1973-174004	19730613
	FR 2190277	A5	19740125	FR 1973-21759	19730614
	CH 573117	A	19760227	CH 1973-8645	19730614
	SE 413326	B	19800519	SE 1973-8385	19730614
	SE 413326	C	19800904		
	BE 801106	A1	19731219	BE 1973-132423	19730619
	JP 49064495	A2	19740621	JP 1973-69109	19730619
	SU 639487	D	19781225	SU 1973-1975398	19731213
PRAI	DE 1972-2229849		19720619		

AB **Triglycerides** are detd. by their enzymic sapon. by a **lipase** from *Rhizopus arrhizus* and measurement of the **glycerol** liberated. The sapon. is performed at pH 6-9 in the presence of carboxylesterase and alkali or alk. earth alkyl sulfate with 10-15 C-atoms in the alkyl group and in the presence of serum albumin. A reagent is composed of 0.1-10.0 mg/ml **lipase** from *R. arrhizus*, 0.5-20.0 mg/ml carboxylesterase, 0.01-0.2 mg/ml Na dodecyl sulfate, 1-20mM NADH, 10-100mM ATP, 0.05-10 mg **glycerol kinase**, 0.1-2.0 mg/ml serum albumin, 0.5-5 mg/ml of lactate dehydrogenase, 2-20mM phosphoenolpyruvate, 0.2-5 mg/ml pyruvate **kinase**, 3-30mM Mg<sup>2+</sup>, and 0.03-0.3M of a pH 6-9 buffer soln. (e.g. triethanolamine buffer).

L21 ANSWER 68 OF 71 CA COPYRIGHT 2003 ACS

AN 78:94577 CA  
 TI **Triglyceride** hydrolysis and assay  
 IN Bucolo, Giovanni; David, Harold  
 PA Calbiochem  
 SO U.S., 4 pp.  
 CODEN: USXXAM  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 3703591	A	19721121	US 1970-98904	19701216
	ES 395956	A1	19740901	ES 1971-395956	19711013
	FR 2118454	A5	19720728	FR 1971-40669	19711112
	CA 955161	A1	19740924	CA 1971-128467	19711124
	IL 38235	A1	19741022	IL 1971-38235	19711126
	CH 563404	A	19750630	CH 1975-2024	19711126

CH 566003	A	19750829	CH 1971-17206	19711126
BE 776034	A1	19720316	BE 1971-111087	19711130
GB 1373106	A	19741106	GB 1971-55609	19711130
AU 7136416	A1	19730607	AU 1971-36416	19711202
BR 7108185	A0	19730531	BR 1971-8185	19711209
IT 972079	A	19740520	IT 1971-54760	19711215
SE 389919	B	19761122	SE 1971-16097	19711215
JP 54009518	B4	19790425	JP 1971-101123	19711215
DE 2162325	B2	19800424	DE 1971-2162325	19711215
DE 2162325	C3	19801211		
NL 7117275	A	19720620	NL 1971-17275	19711216
NL 180523	B	19861001		
NL 180523	C	19870302		
CS 212729	P	19820326	CS 1971-8751	19711216
CA 985607	A2	19760316	CA 1973-188486	19731219
PRAI US 1970-98904		19701216		
CA 1971-128467		19711124		

AB **Glycerol** esters present in aq. media (e.g. serum **triglyceride** and milk fat) are assayed by complete enzymic hydrolysis using both a **lipase** and a protease. The method provides a rapid and accurate procedure for liberation of **glycerol** which can then be detd. The hydrolysis is made in the presence of 3 addnl. enzymes, whereby the liberated **glycerol** is converted to .alpha.-**glycerol** phosphate by **glycerol kinase** with simultaneous conversion of ATP to ADP. The use of pyruvate **kinase** converts the latter back to ATP with the formation of a pyruvate ion. Lactate dehydrogenase then changes pyruvate to lactate with conversion of NADH to NAD. This last change can be detected spectrometrically.

L21 ANSWER 69 OF 71 CA COPYRIGHT 2003 ACS

AN 77:72351 CA

TI Determination of **triglycerides** in blood

IN Bucolo, Giovanni; David, Harold

PA Calbiochem

SO Ger. Offen., 19 pp.

CODEN: GWXXBX

DT Patent

LA German

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2162325		19720622		
PRAI	US 1970-98904		19701216		

AB **Triglycerides** in blood serum were quant. detd. by enzymic hydrolysis of the **triglycerides** by a **lipase**-protease mixt., reaction of **glycerol** formed with ATP by glycerokinase (I) to give **glycerol** 1-phosphate + ADP which reacted with phosphoenolpyruvate in the presence of pyruvate **kinase** (II) to give ATP and pyruvate. Treatment of pyruvate with NADH and lactate dehydrogenase (III) gave lactate and NAD. The amt. of NAD is proportional to the amt. of **triglycerides**. Thus, 3 ml test combination A contained 0.1M K phosphate buffer of pH 7, 1.6 mg Mg aspartate, 0.9 .mu.mole ATP di-Na salt, 0.9 .mu.mole phosphoenolpyruvate, 5.0 mg bovine serum albumin, NAD to final absorption 0.8 (optical d. at 340 nm), 6 IU II, 2 IU III, 39 IU .alpha.-chymotrypsin, and 1200 units **lipase** of *Rhizopus delemar*. Blood serum (30 .mu.l) was added to A, the mixt. incubated 10 min at 25-37.degree., and the optical d. read at 340 nm. The mixt. was treated with 2 IU I, stored in a sep. bottle, kept 10 min, and d. read at 340 nm. The difference of both values is proportional to the content of **triglyceride** in the sample.

L21 ANSWER 70 OF 71 CA COPYRIGHT 2003 ACS

AN 75:85117 CA

TI Enzymic determination of lipoprotein bound **triglycerides** in serum  
 IN Stork, Harald; Schmidt, Felix Helmut  
 PA Boehringer Mannheim G.m.b.H.  
 SO Ger. Offen., 12 pp.  
 CODEN: GWXXBX  
 DT Patent  
 LA German  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	DE 2000127	A	19710708	DE 1970-2000127	19700102
	DE 2000127	B2	19740516		
	DE 2000127	C3	19741212		
	US 3759793	A	19730918	US 1970-100498	19701221
	SU 371734	D	19730222	SU 1970-1607102	19701223
	CH 562450	A	19750530	CH 1970-19271	19701229
	JP 59015638	B4	19840410	JP 1970-130788	19701229
	ZA 7008736	A	19720927	ZA 1970-8736	19701230
	ES 386933	A1	19730401	ES 1970-386933	19701230
	CA 950809	A1	19740709	CA 1970-102122	19701230
	SE 378678	B	19750908	SE 1970-17728	19701230
	NL 7019083	A	19710706	NL 1970-19083	19701231
	NL 166545	B	19810316		
	NL 166545	C	19810817		
	FR 2075161	A5	19711008	FR 1970-47482	19701231
	GB 1295992	A	19721108	GB 1970-1295992	19701231
	AT 303975	B	19721227	AT 1970-11784	19701231
	FI 51744	B	19761130	FI 1970-3527	19701231
PRAI	DE 1970-2000127		19700102		

AB The title **triglycerides** were saponified with *Rhizopus arrhizus* **lipase** having 3500-8000 units/mg activity at pH 3.5-7.0 and the **glycerol** formed was determined enzymically by known methods. Thus, 0.1M (HOCH<sub>2</sub>CH<sub>2</sub>)<sub>3</sub>N (I) at pH 7.6 containing 0.12 mmole of NADH and 0.74 mmole of MgSO<sub>4</sub> 1.5, 0.1M I at pH 7.6 containing 4.8 mmoles of Na phosphoenolpyruvate and 1.3 mmoles of ATP 0.1, 4 mg/ml of *R. arrhizus* **lipase** of 14,000 units/ml 0.02, and a solution containing lactate dehydrogenase of 900 units/ml and pyruvate **kinase** of 150 units/ml 0.005 ml were successively added to 10  $\mu$ l of human serum. After 5 min the absorbance was determined and 0.005 ml of **glycerol kinase** at 85 units/ml was added. After 10 min the absorbance was determined again. From the difference,  $\Delta E$ , the **triglyceride** content was calculated according to the equation  $\Delta E \times 4224 = \text{mg\% triglycerides}$ .

L21 ANSWER 71 OF 71 CA COPYRIGHT 2003 ACS

AN 71:67863 CA

TI Enzymic determination of serum mono-, di-, and **triglycerides** following separation by thin-layer chromatography

AU Zoellner, Nepomuk; Wolfram, H.; Wolfram, Guenther

CS Med. Poliklin., Univ. Muenchen, Munich, Fed. Rep. Ger.

SO Zeitschrift fuer Klinische Chemie und Klinische Biochemie (1969), 7(4), 339-45

CODEN: ZKCKAD; ISSN: 0044-2933

DT Journal

LA German

AB Neutral glycerides were extracted from human plasma, separated by thin-layer chromatography into mono- (MG), di- (DG) and **triglycerides** (TG), and hydrolyzed. The **glycerol** liberated was analyzed by the 3-step glycerokinase- pyruvate **kinase**-lactate dehydrogenase system. The av. ratio MG:DG:TG in the serum of (10) healthy persons (fasted over night) was 3.16:5.66:91.18. TG were significantly increased in hyperlipemia. In alimentary hyperlipemia, heparin-activated lipoprotein **lipase** (I) cause a significant drop of TG with an increase of MG,

L11 ANSWER 22 OF 23 CA COPYRIGHT 2003 ACS  
 AN 74:958 CA  
 TI Rapid method for the isolation of lipoproteins from human serum by precipitation with **polyanions**  
 AU Burstein, Meier; Scholnick, H. R.; Morfin, R.  
 CS Cent. Nat. Transfus, Sanguine, Paris, Fr.  
 SO Journal of Lipid Research (1970), 11(6), 583-95  
 CODEN: JLPRAW; ISSN: 0022-2275  
 DT Journal  
 LA English  
 AB Procedures are described for the isolation of lipoproteins from human serum by pptn. with **polyanions** and divalent cations. A mixt. of low and very low d. lipoproteins can be prepd. without ultracentrifugation by pptn. with heparin and either MnCl<sub>2</sub> alone or MgCl<sub>2</sub> plus sucrose. In both cases the pptn. is reversible, selective, and complete. The highly concd. isolated lipoproteins are free of other plasma proteins as judged by immunol. and electrophoretic methods. The low d. and very low d. **lipoproteins** can then be **sepd.** from each other by ultracentrifugation. The advantage of the method is that large amts. of lipoproteins can be prepd. with only a single preparative ultracentrifugation. **Polyanions** other than heparin may also be used; when the pptn. of the low and very low d. lipoproteins is achieved with dextran sulfate and MnCl<sub>2</sub>, or Na phosphotungstate and MgCl<sub>2</sub>, the high-d. lipoproteins can subsequently be pptd. by increasing the concns. of the reagents. These lipoproteins, contg. small amts. of protein contaminants, are further purified by ultracentrifugation at d. 1.22. With a single preparative ultracentrifugation, immunol. pure high-d. lipoproteins can be isolated from large vols. of serum.  
 CC 6 (Biochemical Methods)  
 ST lipoproteins serum pptn **polyanions**; serum lipoproteins pptn **polyanions**; **polyanions** pptn serum lipoproteins; cations pptn lipoproteins  
 IT Lipoproteins  
 RL: ANST (Analytical study)  
 (blood-serum, isolation by pptn. with **polyanions**)



L7 ANSWER 2 OF 9 CA COPYRIGHT 2003 ACS  
AN 95:38504 CA  
TI Enzymic determination of phospholipids in serum lipoproteins  
**separated** by electrophoresis. I  
AU Takizawa, Akira; Hara, Ichiro; Imai, Toshio  
CS Dep. Chem., Tokyo Med. Dent. Univ., Ichikawa, 272, Japan  
SO Rinsho Byori (1981), 29(4), 414-18  
CODEN: RBYOAI; ISSN: 0485-1404  
DT Journal  
LA Japanese  
AB Serum .alpha.- and .beta.-**lipoprotein fractions**  
**sepd.** on an agarose thin-layer film by electrophoresis were  
reacted with an enzymic reagent consisting of phospholipase, choline  
oxidase, 4-aminoantipyrine, and PhOH and detd. by densitometry. The total  
serum phospholipid and .alpha.-to-.beta. ratio agree with those detd. by a  
com. kit prepd. on the basis of enzymic detn. and by **polyanion**  
pptn. method with heparin-Mn reagent.

L10 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS  
 AN 97:212064 CA  
 TI Colorimetric triglyceride determination  
 PA Wako Pure Chemical Industries, Ltd., Japan  
 SO Jpn. Kokai Tokkyo Koho, 7 pp.  
 CODEN: JKXXAF  
 DT Patent  
 LA Japanese  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 57137858	A2	19820825	JP 1981-23745	19810220 <--
PRAI	JP 1981-23745		19810220		

AB For triglycerides detn., free glycerol and reducing substances present in a biol. sample are completely decompd. with HIO<sub>4</sub> (0.0005-0.003M) at pH 0-3.5, and then triglycerides in the sample are enzymically hydrolyzed into glycerol and fatty acids. The glycerol formed quant. is detd. to est. the triglyceride content in the sample. Thus, 20 .mu.L serum and 0.6 mL HIO<sub>4</sub>-2H<sub>2</sub>O (0.04 g/100 mL) were mixed, incubated at 37.degree. for 3 min, and to this was added 2.5 mL soln. contg. lipoprotein lipase 3500, glycerokinase 200, glycerol 3-phosphate oxidase 120, peroxidase 200 units, Na ATP 100, 4-aminoantipyrine 9, p-chlorophenol 70 mg, Mg(OAc)<sub>2</sub> 4 mM, Triton X405 20 mg in 100 mL 0.05M Tris buffer. The mixt. was incubated at 37.degree. for 10 min and the color developed was measured at 505 nm.

IC G01N033-52; C12Q001-00; G01N033-50  
 CC 9-2 (Biochemical Methods)  
 ST serum triglyceride detn; enzymic detn triglyceride; colorimetry triglyceride detn  
 IT Glycerides, analysis  
 RL: ANT (Analyte); ANST (Analytical study)  
 (detn. of, in human blood serum, enzymic-colorimetric)  
 IT Blood analysis  
 (triglycerides detn. in, of human, enzymic-colorimetric)  
 IT Spectrochemical analysis  
 (spectrophotometric, for triglycerides, of human blood serum)  
 IT 9004-02-8 13444-71-8  
 RL: ANST (Analytical study)  
 (in triglycerides enzymic-colorimetric detn. in human blood serum)

=>

L6 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS  
 AN 125:216395 CA  
 TI Method of quantitative analysis of cholesterol  
 IN Hino, Kouichi; Nakamura, Mitsuhiro; Manabe, Mitsuhiisa  
 PA Daiichi Pure Chemicals Co., Ltd., Japan  
 SO PCT Int. Appl., 11 pp.  
 CODEN: PIXXD2

DT Patent  
 LA Japanese

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9623902	A1	19960808	WO 1995-JP641	19950403
	W: AU, CA, CN, KR, MX, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	JP 08201393	A2	19960809	JP 1995-13607	19950131 <--
	JP 2799835	B2	19980921		
	CA 2185562	AA	19960808	CA 1995-2185562	19950403
	AU 9520852	A1	19960821	AU 1995-20852	19950403
	AU 696681	B2	19980917		
	EP 753583	A1	19970115	EP 1995-913411	19950403
	EP 753583	B1	20001018		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	CN 1145096	A	19970312	CN 1995-192343	19950403
	CN 1072724	B	20011010		
	AT 197070	E	20001115	AT 1995-913411	19950403
	ES 2153030	T3	20010216	ES 1995-913411	19950403
	TW 400385	B	20000801	TW 1995-84103384	19950408
	<u>US 5773304</u>	A	19980630	US 1996-704681	19960919
PRAI	JP 1995-13607	A	19950131		
	WO 1995-JP641	W	19950403		

AB Disclosed is a method of quant. anal. of cholesterol contained in a high-specific-gravity lipoprotein by adding to a lipoprotein-contg. specimen surfactant and a substance which forms a complex with a lipoprotein other than the high-specific-gravity lipoprotein and a surfactant, and enzymically detg. the cholesterol content. The complex-forming substance is a polyanion, divalent ion, sol. polymer, or antibody specific to lipoprotein other than high-specific-gravity lipoprotein. The enzymic anal. uses cholesterol esterase in combination with cholesterol oxidase. The method permits the quant. anal. of cholesterol contained in a high-specific-gravity lipoprotein with a simple operation at a high efficiency without the necessity for pre-treatment such as centrifugal sepn. This method is applicable to various automatic analyzers and useful also in the field of clin. examn. In example, the method was used to quantitate cholesterol content in HDL.

